III.

REVIEW OF LITERATURE
### III. REVIEW OF LITERATURE

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Title</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Historical Milestones</td>
<td>6-9</td>
</tr>
<tr>
<td>2</td>
<td>Habitat</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Bacteriology</td>
<td>11-25</td>
</tr>
<tr>
<td>4</td>
<td>Pathogenesis and Virulence Factors</td>
<td>26-46</td>
</tr>
<tr>
<td>5</td>
<td>Clinical Infections</td>
<td>47-56</td>
</tr>
<tr>
<td>6</td>
<td>Epidemiology</td>
<td>57-79</td>
</tr>
<tr>
<td>7</td>
<td>Laboratory Diagnosis</td>
<td>80-92</td>
</tr>
<tr>
<td>8</td>
<td>Antibiotic Therapy</td>
<td>93-107</td>
</tr>
<tr>
<td>9</td>
<td>Emergence of Antimicrobial Resistance</td>
<td>108-132</td>
</tr>
<tr>
<td>10</td>
<td>Prevention and Control</td>
<td>133-146</td>
</tr>
</tbody>
</table>
3. REVIEW OF LITERATURE

HISTORICAL MILESTONES

Earlier documented microorganisms such as ‘streptococci of faecal origin’ or ‘enterococci’ are now included in the genus *Enterococcus*. The term ‘enterococci’ probably originated with the discovery of the first organism of this group.\(^{[12]}\)

1899: Thiercelin described the bacteria as seen in pairs and short chains in human faeces. MacCallum and Hasting described a case of acute endocarditis caused by bacterium that was ‘very hard and tenacious life’, which was named *Micrococcus zymogens*. A few years later Gordon reported, the isolation of the faecal streptococci in broth exposed to the air probably contaminated with animal faeces.\(^{[12]}\) Houston (1997) noted the abundance of streptococci in sewage, proposing that they might be useful for detecting contamination of water by human faeces. The name *Enterococcus* is derived from a paper published in France, he described an “enterocoque” a new Gram-positive cocci of intestinal origin.\(^{[1]}\)

1906: Andrews and Horder used the name *Streptococcus faecalis* (*faecalis* related to faeces) to identify an organism of faecal origin that clotted milk and fermented mannitol and lactose but not raffinose.\(^{[12]}\)

1919: Orla-Jensen described a second organism that is *Streptococcus faecium*, which differed from the fermentation patterns of *Streptococcus faecalis*.\(^{[12]}\)

1930: Lancefield established the serological typing system to differentiate enterococci (group D) from streptococci.\(^{[27]}\)

1934: Todd was the first to study systematically the virulence factor of enterococci such as cytolysin or haemolysin.\(^{[20]}\)
1935 & 37: Sherman and Wing proposed a third species, *Streptococcus durans*, which was similar to *Streptococcus faecium* but had less fermentation activity.\textsuperscript{[12,19,27]}

1938: Sherman used the term ‘enterococcal group’ to describe streptococci that grew at 10\(^0\) C and 45\(^0\) C, in broth with pH 9.6 and broth containing 6.5% NaCl and survived at 60\(^0\) C for 30 minutes.\textsuperscript{[12]}

1947: Penicillin- streptomycin synergy was first demonstrated against enterococci.\textsuperscript{[3]}
1952 & 64: Sharpe, Shattock & Barnes showed the presence of carbohydrate type antigen on the cell surface of *E. faecalis*, *E. faecium* and *E. durans*.\[12\]

1955: Schultz-Haudt and Scherp postulated that the initial lesion in periodontal disease might be due to disruption of intercellular cementing substances of the epithelium because of mucopolysaccharidase (or hyaluronidase) activity of *E. faecalis*.\[20\]

1962 & 64: Shattock and Deibel presented the classification scheme for group D streptococci that included two non-enterococcal group D streptococci, *Streptococcus bovis* and *Streptococcus equinus*.\[43\] Shattock, Deibel and Hartman *et al.* have also included *Streptococcus faecium* as distinct species. The terms ‘faecal streptococci’, ‘enterococci’ and ‘group D streptococci’ have been discussed by Hartman *et al.*\[36\]

1964: Barnes observed the bright red center of colony on agar containing tetrazolium salts.\[12\]

1967: Nowlan and Deibel added *Streptococcus avium* to the enterococcal group.\[12\]

1969: Toala *et al.* speciated the enterococci and included *S. faecium* in their identification procedures.\[43\]

1970: Kalina proposed that a genus *Enterococcus* for the faecal streptococci based on cellular arrangement and phenotypic characteristics, and *Streptococcus faecalis* and *Streptococcus faecium* be named *Enterococcus faecalis* and *Enterococcus faecium*.\[12,40\]

1970: Standiford HD reported enterococcal resistance to streptomycin or kanamycin and MICs were >2,000 µg/ml (high-level aminoglycoside resistance).\[29\]

1971: Sabbaj *et al.* observed the grey-white colonies surrounded by black halo on media containing esculin (such as bile esculin azide medium & enterococcusel agar).\[12\]

1972: Facklam and Collins published 26 physiologic tests to differentiate group D streptococci in to different species.\[44\]

1975: Gross *et al.* used three tests (pyruvate utilization, deamination of arginine, and acidification of sorbose broth) for identification of *Enterococcus* species and documented the occurrence of *S. avium* in humans.\[44\]

1978: Sex pheromone system of *E. faecalis* was described.\[45\]
1979: Horodniceanu reported first time high-level gentamicin resistance (HLGR, MIC $>1000$ mg/L) in *Enterococcus faecalis* from France.$^{[29,30,46]}$
1980: Enterococci were removed from the genus *Streptococcus* and placed in their own genus *Enterococcus*, based on their genetic differences from streptococci.\(^{[27]}\)

1983: Penicillinase production in enterococci was described by Murray and Mederski-Samoraj first time.\(^{[47]}\)

1983: De Vriese and his associates described *Streptococcus cecorum*, a group of carboxyphilic strains isolated from the caeca of chickens.\(^{[48]}\)

1984: The genus *Streptococcus* was split into three genera, *Enterococcus*, *Lactococcus* and *Streptococcus*. The justification and background for this action was reviewed by Schleifer and Kilpper-Balz. DNA-DNA hybridization studies have confirmed various *Enterococcus* species such as *E. avium*, *E. durans*, *E. casseliflavus*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. raffinosus* and *E. solitarius*.\(^{[11,31,49]}\)

1986: Vancomycin resistant enterococci (VRE) were first detected in Europe and soon after a VanB *E. faecalis*, clinical isolate was reported from St. Louis in the United States.\(^{[25]}\)

1987 & 2002: Schleifer, Kilpper-Blaz and more recently Coyette and Hancock studied enterococcal cell wall chemistry.\(^{[12]}\)

1988: Uttley *et al.* were reported the vancomycin resistant *E. faecalis* and *E. faecium* first time in England.\(^{[27]}\)

1989 & 1991: Guzman *et al.* reported the evidence indicating the existence of carbohydrate adhesins in enterococci.\(^{[20]}\)

1993: Murray tested *E. faecalis* for various growth requirements.\(^{[12]}\)

1993: Shorrock and Lambert investigated the binding of fibronectin and albumin by *E. faecalis*.\(^{[20]}\)

1993: Fraimow first time reported vancomycin dependent *E. faecalis*.\(^{[50]}\)

1995 & 97 and 2001: Facklam, Elliott & Teixeira and Barrow demonstrated the lipoteichoic acid (antigen) in the strains belonging to the *Enterococcus species*, *Streptococcus bovis* complex, as well as *Leuconostoc, Pediococcus and Vagococcus*.\(^{[12]}\)

1999: Quinupristin-dalfopristin was introduced to the US market.\(^{[28]}\)

2002: Huycke emphasized the physiology of enterococci and he described respiration, ion transport, pyrimidine and foliate pathway and tress responses.\(^{[12]}\)
Proposal of species to be included in the genus *Enterococcus*[^12]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Year</th>
<th><em>Enterococcus</em> species</th>
<th>Name of scientist</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>1984</td>
<td><em>Enterococcus faecalis</em></td>
<td>Schleifer and Kilpper-Blaz</td>
</tr>
<tr>
<td>02.</td>
<td>1984</td>
<td><em>Enterococcus faecium</em></td>
<td>Schleifer and Kilpper-Blaz</td>
</tr>
<tr>
<td>03.</td>
<td>1984</td>
<td><em>Enterococcus avium</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>04.</td>
<td>1984</td>
<td><em>Enterococcus casseliflavus</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>05.</td>
<td>1984</td>
<td><em>Enterococcus durans</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>06.</td>
<td>1984</td>
<td><em>Enterococcus gallinarum</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>07.</td>
<td>1984</td>
<td><em>Enterococcus malodoratus</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>08.</td>
<td>1985</td>
<td><em>Enterococcus hirae</em></td>
<td>Farrow and Collins.</td>
</tr>
<tr>
<td>09.</td>
<td>1986</td>
<td><em>Enterococcus mundii</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>10.</td>
<td>1989</td>
<td><em>Enterococcus raffinosus</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>11.</td>
<td>1989</td>
<td><em>Enterococcus solitarius</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>12.</td>
<td>1989</td>
<td><em>Enterococcus pseudoavium</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>13.</td>
<td>1989</td>
<td><em>Enterococcus cecorum</em></td>
<td>Williams <em>et al.</em></td>
</tr>
<tr>
<td>14.</td>
<td>1990</td>
<td><em>Enterococcus columbae</em></td>
<td>Devriese <em>et al.</em></td>
</tr>
<tr>
<td>15.</td>
<td>1990</td>
<td><em>Enterococcus saccharolyticus</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>16.</td>
<td>1991</td>
<td><em>Enterococcus dispar</em></td>
<td>Collins <em>et al.</em></td>
</tr>
<tr>
<td>17.</td>
<td>1991</td>
<td><em>Enterococcus sulfureus</em></td>
<td>Martinez-Murcia &amp; Collins <em>et al.</em></td>
</tr>
<tr>
<td>18.</td>
<td>1991</td>
<td><em>Enterococcus seriolicida</em></td>
<td>Kusuda <em>et al.</em></td>
</tr>
<tr>
<td>19.</td>
<td>1992</td>
<td><em>Enterococcus flavescens</em></td>
<td>Pompei <em>et al.</em></td>
</tr>
<tr>
<td>20.</td>
<td>1998</td>
<td><em>Enterococcus asini</em></td>
<td>De Vaux <em>et al.</em></td>
</tr>
<tr>
<td>21.</td>
<td>2001</td>
<td><em>Enterococcus villorum</em></td>
<td>Vancanneyt <em>et al.</em></td>
</tr>
<tr>
<td>22.</td>
<td>2001</td>
<td><em>Enterococcus haemoperoxidus</em></td>
<td>Svec <em>et al.</em></td>
</tr>
<tr>
<td>23.</td>
<td>2001</td>
<td><em>Enterococcus moraviensis</em></td>
<td>Svec <em>et al.</em></td>
</tr>
<tr>
<td>24.</td>
<td>2001</td>
<td><em>Enterococcus ratti</em></td>
<td>Teixeira <em>et al.</em></td>
</tr>
<tr>
<td>25.</td>
<td>2001</td>
<td><em>Enterococcus porcinus</em></td>
<td>Teixeira <em>et al.</em></td>
</tr>
<tr>
<td>26.</td>
<td>2002</td>
<td><em>Enterococcus gilvus</em></td>
<td>Tyrrell <em>et al.</em></td>
</tr>
<tr>
<td>27.</td>
<td>2002</td>
<td><em>Enterococcus pallens</em></td>
<td>Tyrrell <em>et al.</em></td>
</tr>
<tr>
<td>29.</td>
<td>2003</td>
<td><em>Enterococcus canis</em></td>
<td>De Graef <em>et al.</em></td>
</tr>
</tbody>
</table>
Several intrinsic characteristics of the enterococci allow them to grow and survive in harsh environments and to persist almost everywhere. Enterococci tolerate hypotonic, hypertonic and alkaline environments. They are widespread in nature and can be found in soil, water (contaminated), plants, food especially dried milk, cheddar, artisanal cheese and meat.

Enterococci species inhabit the gastrointestinal tract of warm-blooded animals, including mammals, birds as well as reptiles and insects. Therefore, these bacteria are useful indicators of faecal contamination in water and foods. Human, poultry, cattle, pigs, dogs, horses, donkey, sheep, goats, rabbits, rats and parrot are natural reservoirs. The prevalence of certain species of enterococci in intestinal tract of various animals, are implicated in the spoilage of meat and pork products.

Enterococci are normal residents of the human gastrointestinal tract in higher numbers. There are often $10^5$ to $10^7$ colony forming units per gram of stool sample in 80% of hospitalized patients. They are occasionally present in biliary tracts and in lesser numbers are found in the mouth, vagina (17%), male urethra and on the skin of healthy individuals especially in the perineal area. E. faecalis is most common species. Other enterococcal species such as E. faecium (up to 30% of hospitalized patients) as well as E. durans, E. casseliflavus, E. gallinarum and E. cecorum have also been found in variable proportions. Prevalence of different enterococcal species appears to vary according to the hosts and influenced by age, diet and other factors that may relate to change in physiological conditions such as underlying diseases and prior antimicrobial therapy.

Some species like E. casseliflavus have been recovered from plants, soil, rarely from faeces of chicken. E. mundtii is also isolated from plant, soil and the gastrointestinal tracts of cattle. E. ratti were isolated from gastrointestinal tract of rats. E. haemoperoxidus is a new environmental enterococcal species, isolated from surface water, swimming pool and drinking water. E. asini is found in the donkey. E. cecorum an intestinal commensal, is isolated from chickens, pigs, calves, horses, ducks, cats, dogs and canaries. E. sulfureus is isolated from plants. E. columbae dominates the intestinal flora of domestic pigeons and E. seriolicida is a fish pathogen. E. hirae has been isolated from rabbit, human, cattle, chicken and hog intestines.
Enterococci are Gram positive cocci; oval or coccobacillary in shape and 0.6 - 2.0 x 0.6 - 2.5 µm in size. They occur singly or arranged in pairs or short chains. They are more oval and found in chains when Gram stain is done from thioglycolate broth.\cite{49} Mostly, they are attached in angle to one another, so chains are typically curved. As such, they are difficult to distinguish morphologically from streptococci.\cite{3,59} Most of the species are non-motile, non-capsulated and non-acid fast; while \textit{E. casseliflavus} and \textit{E. gallinarum} are motile by the presence of
Cell Wall Composition and Antigenic Structure:

The enterococcal cell wall consists of peptidoglycan, glucose-glycosyl glycerol,[59] teichoic acid, polysaccharide and proteins. Peptidoglycan polymers are the main structure and shape maintaining components of the cell wall. The mesh of the peptidoglycan net is made of parallel glycan chain held together by small peptide cross-links. The glycan moiety is composed of alternating β-1, 4-linked units of N-acetylglucosamine (Glu-Nac) and N-acetylmuramic acid (Mur-Nac). Short-stem peptides connect to glycan chains through amide linkages between the carboxyl group of muramyle residues and terminal amino group of peptides. The sequence of the enterococcal stem peptide is L-Ala-D-Ala-Glu-L-Lys-D-Ala. The D-glutamic acid is bound through its amino group to the L-Ala carboxyl group and its γ-carboxyl group to the amino group of the L-Lys residue. Adjacent stem peptides are cross-linked by interpeptide bridges.[12]

Chemical analysis showed that the group D antigen is a lipoteichoic acid. So-called ‘Streptococcal group D antigen’ is usually associated with Enterococcus species, but it is not exclusive. This antigen is found in the strains belonging to the
Streptococcus bovis complex, Leuconostoc, Pediococcus and Vagococcus. The carbohydrate substituent of teichoic acids can act as antigen. Davie and Brock have suggested that the D-alanine in the teichoic acid of group D streptococci inactivates an autogenous lytic factor. The autolytic enzyme of group D streptococci is a muraminidase. The group D, type I antigenic determinant is a-D-glucose or a-D-N-acetylglucosamine substituted on a phosphorylated heteropolymer containing ribitol, glucosamine, galactosamine, glucose and rhamnose. Carbohydrate antigens are also present on the surface of the cells of strains of E. faecalis, E. faecium and E. durans.

Metabolism and Growth Requirements:

The nutritional requirements of the enterococci are complex and microorganisms recognised by their metabolic adaptability in order to fulfil diverse role as commensals and as pathogens. The capacity to utilize the variety of carbohydrates as well as characteristic tolerance to a diversity of physical and chemical conditions, including pH, temperature, salt concentrations, detergents and antimicrobial compounds are likely to enhance enterococcal competitiveness in many harsh environments.

More recently, Huycke (2002) emphasized the physiology of enterococci and described central carbon and oxygen metabolism, respiration, ion transport, pyrimidine and folate pathway, stress responses. Most studies addressing the growth requirements and physiology of the enterococci have been done on E. faecalis strains. Growth requirements include vitamin B, nucleic acid bases, carbon source and glucose. E. faecalis requires histidine, isoleucine, methionine and tryptophan while some strains require arginine, glutamate, glycine, leucine and valine (Murray et al., 1993). Intensive antimicrobial pressures have created atypical growth requirements, such as vancomycin requiring strains. The different species and strains of enterococci have different growth requirements.

Cultural Characteristics:

Enterococci are aerobic and facultative anaerobic micro-organisms. They grow
under reduced or oxygenated environments. They grow readily on ordinary nutrient media, enriched blood agar as well as MacConkey’s agar and other selective media.\textsuperscript{[12,13,14,58]} Though, they are not capable of forming spores, they tolerate wide range of environmental conditions and has the ability to grow in following extreme conditions:\textsuperscript{[3,7,11,44,60,61]}
Review of literature [Bacteriology] 2015

i. 40 percent bile
ii. 6.5% sodium chloride
iii. More alkaline pH i.e. 4.5 to 10
iv. Wider range of temperatures (10^0-45^0C)
v. Many of them can survive at 60^0 C for 30 minutes

**Colony Characteristics:**

**Nutrient agar:**

Colonies after 24 to 48 hours of incubation

- Size: 0.5 to 2 mm in diameter
- Shape: Spherical in shape
- Edges: Entire
- Surface: Smooth
- Elevation: Elevated or convex
- Opacity: Partially opaque
- Consistency: moist or emulcifiable
- Colour: white or cream

**MacConkey’s agar:** It usually produces tiny magenta (deep pink) coloured colonies due to lactose fermentation.[13]

**Cystine Lactose Electrolyte Deficient media:** It produces yellow coloured colonies.[13]

**Blood agar (Haemolysis):**

All species are non-haemolytic or α- haemolytic. Some (about one-third) cultures of *E. faecalis* may be β haemolytic on blood agar containing rabbit, horse, cow or human blood but non-haemolytic on blood agar containing sheep blood. This difference is because the target cells i.e. sheep RBC’s are refractory to cytolysin mediated lysis.[20] Strains that appear α- haemolytic are actually non-haemolytic but haemolysis is because of production of peroxidase. The ‘greening’ of the agar is due to production of peroxidase enzyme which acts on red blood cells in the medium and not due to production of α- toxin.[12]

**Pigment production:**

*E. casseliflavus,[62] E. mundtii,[63] E. gilvus, E. pallens* and *E. sulfureus* produce a yellow pigment. Inoculate the organism on trypticase soya agar and after overnight incubation, by using a cotton swab pick up the growth and examine the swab for yellow coloured pigment.[44] Pigment production also investigated on Columbia agar with 5% sheep blood.[64]
Colonies on selective media:

The colonies of enterococci on selective media will depend on the chemical used in the medium. For example, media containing esculin, (such as bile esculin azide
medium and enterococcusel agar) the colonies will appear grey-white surrounded by a black halo; whereas on agar containing tetrazolium salts the center of the colony will appear bright red.

**Selective media:**

Sodium azide, carbohydrates, antibiotics such as kanamycin or gentamicin, bile salts, an indicator esculin or tetrazolium are used as a selective agents in the medium.\(^4,9,65\)

**Most often used selective media are:**

1. Bile esculin azide agar
2. Kenner faecal *Streptococcus* broth
3. Selective media with vancomycin for VRE
4. Kanamycin esculin azide agar
5. Eosin methylene blue agar
6. Phenyl ethyl alcohol agar
7. Pfizer’s enterococcal selective media

**Biochemical Reactions:**

Biochemical tests used for identification of *Enterococcus* species

1. Catalase test
2. Heat tolerance test
3. Alkaline pH tolerance test
4. Salt tolerance test
5. Bile esculin hydrolysis test
6. Pyrrolidonyl β naphthylamide hydrolysis (PYR) test
7. Arginine hydrolysis
8. Voges-Proskauer (VP)
9. Potassium tellurite reduction test (0.04%)
10. Hippurate hydrolysis
11. Pyruvate fermentation (utilisation)
12. LAPase
13. Litmus milk test
14. Carbohydrate / sugar fermentation tests

**1. Catalase test:**

Like streptococci, enterococci also do not have cytochrome enzymes and are thus catalase negative, although some strains occasionally produce pseudo-catalase.\(^12,19\)

**2. Heat tolerance test:**

Ayers and Johnson (1914) showed thermal resistance of certain kinds of streptococci to pasteurisation. Houston *et al.* (1918) recognized heat tolerance as a physiologic characteristic of enterococci, which is used to differentiate these organisms from other streptococci. Diebel (1921) used this property of enterococci to isolate these
organisms from mixed culture.\textsuperscript{13}
Sherman JM and co-workers (1937) showed the heat tolerance property. The high survival rate of all kinds of streptococci subjected to 60°C for 30 minutes were variable. This could be either due to the methodologies of the heat test or the medium in which test was conducted. The methodology of the heat tolerance test has not been standardized. Some laboratories heat a sample of broth culture to 60°C for 30 minutes and then streak the culture on blood agar plate. Other laboratories use 62.5°C. The size of the inoculum used for the test has not been standardized.

3. Alkaline (pH) tolerance test:

Enterococci are distinguished from streptococci and related taxa by their ability to grow at alkaline (9.6) pH.

4. Salt tolerance test:

Wie Shing Lee used a modified NaCl broth called D broth and produced results within 24 hours. Quadri et al. used a modified conventional medium of Facklam, by using 1% dextrose instead of 0.1% and obtained the results within 8 to 24 hours.

5. Bile esculin hydrolysis test:

First described in 1926 by Meyer and Schonfeld, the bile esculin test was shown by Facklam and Moody to have a sensitivity of 100% and a specificity of 97% for identifying enterococci and group D streptococci.

6. PYR test (Hydrolysis of L-pyrrolidonyl beta-naphthylamide test):

The PYR test was first described by Facklam and co-authors in 1982 and since that time, it has gained acceptance as a rapid test for the presumptive identification of both group A β-haemolytic streptococci and enterococci and to differentiate from pediococci (negative). While the original test described was a 16-20 hour agar test, subsequent PYR test formats include a 4 hours broth assay and several rapid (10-15 min) tests where the PYR reagent is impregnated on filter paper disc or strips on which colonies of test organisms are smeared on it. In 1997, Chi-Hsiang Chen et al. described
a 2 min test format for this test.\textsuperscript{[19,69]}
The substrate used for the PYR test is L-pyrrolidonyl beta naphthylamide. This compound is hydrolysed by a specific bacterial aminopeptidase enzyme. Hydrolysis of the substrate by this enzyme releases free beta naphthylamide, which is detected by the addition of N, N-dimethyl aminocinnamaldehyde. This detection reagent couples with the naphthylamide to form a red schiff base. This test can be done with PYR broth (Todd Hewitt broth with 0.01% L-pyrrolidonyl beta naphthylamide) and PYR reagent (0.01% P-dimethyl amino cinnamaldehyde). After 16-20 hours of incubating the test organism in PYR broth, addition of PYR reagent will lead to the development of cherry red colour, which is considered as positive and development of yellow or orange colour is considered as negative reaction.\textsuperscript{[19,70]}

Positive control- Enterococcus faecalis Negative control - Streptococcus agalactiae.

7. Arginine dihydrolase Test:

Hills first described the hydrolysis of arginine by streptococci. Niven \textit{et al.} reported the deamination of arginine by all enterococci and Gale observed the ability of this amino acid to serve as an energy source in the active uptake of certain amino acids into the amino acid pool. In 1955, Moeller developed the amino acid decarboxylase media to detect the production of arginine dihydrolase. Subsequently Ewing, Davis and Edwards compared Moeller’s medium to Falkow’s decarboxylase medium and they recommended that Moeller’s method as the standard or reference method for determining decarboxylation reactions.\textsuperscript{[19]} In 1975, Gross K. \textit{et al.} concluded that arginine hydrolysis could be used as a dependable test for speciation of enterococci.\textsuperscript{[25]}

8. Voges-Proskauer (VP) test:

In 1987, Fertally SS and Facklam RR tested certain strains of enterococci, for the production of acetyl methyl carbinol and compared the results with rapid strep system results. They described all the four methods of VP tests (Coblentz, Barritt’s, O’Meara’s, Barry and Feeney’s methods) and concluded that Coblentz method will give the best results and thus helps in differentiating the species of enterococci.\textsuperscript{[69]}

9. Potassium Tellurite (0.04%) Reduction:

Diebel in 1964 used 0.05% potassium tellurite reduction as one of the test to
differentiate Enterococci.\textsuperscript{[25]} Facklam in 1971 and in 1989 employed 0.04% potassium tellurite in heart infusion agar with defibrinated rabbit blood as one of the batteries of
tests to speciate the strains of enterococci. Black colonies due to reduction of tellurite to tellurium after any time interval up to three days is taken as positive.[43,44]

10. Hippurate hydrolysis:

Along with group B streptococci, *Enterococcus faecalis* and some other species of enterococci (not *E. faecium*) hydrolyse sodium hippurate to its components, glycine and benzoic acid.[19]

11. Pyruvate fermentation:

Utilisation of pyruvic acid is tested in 1% pyruvate broth with bromothymol blue indicator. *Enterococcus faecalis* (positive) can be differentiated from *E. faecium* (negative) by using this test.[44]

12. Lucien aminopeptidase (LAPase):

LAPase is a useful test to differentiate enterococci (positive) from leuconostocs (negative).[68]

13. Litmus milk:

This test is useful to differentiate new species like *Enterococcus durans* (positive) from *Enterococcus villorum* (negative).[13]

14. Carbohydrate/Sugar fermentation tests:

Andrew in 1900 showed mannitol fermentation as one of the criteria for the classification of enterococci. A wide range of carbohydrates like arabinose, maltose, sucrose, lactose, trehalose, raffinose, inulin, glycerol, mannitol, sorbitol and salicin along with the other tests were used to speciate enterococci.[25,44]

In 1972, Facklam R.R., presented a summary of 26 physiological tests to differentiate the species of enterococci.[71] With this scheme, *E. avium*, *E. faecium*, *E. casseliflavus* or *E. equinus* species were identified. In 1975, Gross et al. incorporated pyruvate utilization, deamination of arginine and acidification of sorbose broth tests to the above scheme, to document the occurrence of *Streptococcus avium* in human beings.[12,44] Subsequently in 1989, Facklam R.R. and Collins M.D. incorporated the above mentioned three tests, along with other 14 tests (from the scheme of Facklam R.R. in 1972) to present a species identification scheme by conventional tests.[12]
Fermentation test is carried out with 1% sugar in brain heart infusion broth with bromocresol purple indicator or peptone water with Andrade’s indicator. \cite{44,72,73} Enterococci ferments sugars without producing of gas, they do not produce gas because
they lack Kreb’s cycle and respiratory chain. Simplified scheme for species identification is mentioned in the flow chart (Page No. 81, 82).

**Classification and Identification:**

Shattock (1962) and Deibel (1964) had described the classification for group D streptococci. Hartman *et al.* discussed the terms "faecal streptococci," "group D streptococci" and "enterococci". These terms are not synonymous to each other. The term *faecal streptococci* have no definitive meaning and should not be used. Various investigators use the term to describe many different streptococcal species of faecal origin. **Group D streptococci** are defined as all those streptococci possessing the group D antigen. This includes all the enterococcal species plus *S. bovis* and *S. equinus*. The *enterococci* include *S. faecalis* and its varieties (zymogenes and liquefaciens), *S. faecium* and *S. durans*. It is probably advisable to retain the use of the term *Enterococcus* because of the difference in antibiotic therapy for patients with enterococcal infections and those with nonenterococcal infections.[43]

The current Bergey's Manual of Systematic Bacteriology lists seven genera of facultative anaerobic, Gram positive cocci. Out of these five genera (including *Streptococcus*) do not contain cytochrome enzymes and are thus catalase negative.[74]

Earlier classification schemes (before 1962) did not include the species *S. faecium*; Shattock, Deibel and Hartman *et al.* have also included *S. faecium* also in their classification schemes. They evidently reported that *S. faecalis* and *S. faecium* are separate species. The inclusion of *S. bovis* and *S. equinus* into the group D classification based on the demonstration of the group D antigen, in extracts of cells of these two species. Toala *et al.* (1969) had speciated the enterococci and included *S. faecium* in their identification procedures. However, they did not include the two non-enterococcal species (*S. bovis* and *S. equinus*) in their identification scheme.[43]

In 1970 Kalina proposed that a genus for the enterococcal streptococci be established and suggested that based on cellular arrangement and phenotypic characteristics, *Streptococcus faecalis* and *Streptococcus faecium* be named *Enterococcus*. [19] But no action on this proposal was ever taken and the use of the genus
name *Streptococcus* continued. For a long time, the enterococci remained the major division of genus *Streptococcus* differentiated by their higher resistance to chemical and physical agents and accommodating most of the serological group D streptococci.
In 1984, the genus *Streptococcus* was divided into three genera, *Streptococcus*, *Lactococcus*, and *Enterococcus*. The taxonomy of *Enterococcus* has undergone significant change since the mid of 1980s. Before the advent and widespread use of genetic techniques for taxonomic analysis, enterococci were distinguished from streptococci and related taxa by their ability to grow at 10°C and 45°C, growth in presence of 6.5% NaCl, growth at pH 9.6, ability to hydrolyze esculin in presence of 40% bile and production of pyrrolidonyl arylamidase (PYR). More than 90% of strains also possessed the Lancefield group D lipoteichoic antigen in their cell walls.

**Percentages of positive reactions of tests for identification of enterococci**

<table>
<thead>
<tr>
<th>Test</th>
<th>Enterococcus</th>
<th>Lactococcus</th>
<th>Leuconostoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive cocci</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Group D antigen</td>
<td>77</td>
<td>00</td>
<td>17</td>
</tr>
<tr>
<td>Bile-esculin reaction</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Growth in 6.5% NaCl broth</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vancomycin susceptibility</td>
<td>99</td>
<td>100</td>
<td>00</td>
</tr>
<tr>
<td>Gas from glucose</td>
<td>&lt;1</td>
<td>00</td>
<td>100</td>
</tr>
<tr>
<td>Pyrrolidonylarylaminidase</td>
<td>100</td>
<td>33</td>
<td>00</td>
</tr>
</tbody>
</table>

Schleifer and Kilpper-Blaz (1984) suggested the establishment of the genus *Enterococcus*, initially, which composed only two species i.e. *E. faecalis* and *E. faecium*. Several other *Enterococcus* species have been described and proposed to be transferred to the new genus. A DNA-DNA reassociation experiment, which is considered the gold standard for establishing the bacterial identification to species level, is carried out for most of the enterococcal species. Sequencing of the 16S rRNA gene is also a very powerful tool in adding the description of bacterial species. This procedure is not recommended for defining new species. Nevertheless, together with the result of DNA-DNA reassociation experiments or whole cell-protein analysis and phenotypic characterisation it can be very useful, especially in relating one species to another. Gene Sequencing of the 16S rRNA has been performed on all enterococcal species.[12]

The catalase negative Gram positive coccii of human origin are now classified among six families and in the proposed order “Lactobacillales”. Moreover, enterococci are classified as: [11,12]
Kingdom - Protista
Sub Kingdom - Bacteria
Phylum (division) - Firmicutes
Class - Bacilli
Order - Lactobacillales
Family - Enterococcaceae
Genus - Enterococcus.

Species: *E. faecalis*, *E. faecium*, *E. avium*, *E. casseliflavus*, *E. cecorum*, *E. durans*, *E. hirae*, *E. gallinarum*, *E. mundtii*, *E. dispar*, *E. malodoratus*, *E. pseudoavium*, *E. raffinosus*, *E. saccharolyticus*, *E. seriolicida*, *E. solitarius*, *E. columbae*, *E. sulfureus*, *E. asini*, *E. haemoperoxidus*, *E. gilvus*, *E. pallens*, *E. bovis*, *E. phoeniculicola*, *E. moraviensis* & *E. species CDC PNS-E1, E2 & E3* (*Are not isolated from human beings*).

Identification & classification of enterococci to species level is useful for proper patient treatment, epidemiological study and infection control purpose. Isolates of *E. faecium* tend to be more resistant to penicillin, ampicillin and vancomycin than *E. faecalis* isolates.

Identification of *Enterococcus species* is accomplished by using key phenotypic characteristic (biochemical and physiological tests) to form groups at the species level. Most (80%) *Enterococcus species* react with antiserum against Lancefield group D. Remaining species of enterococci do not contain the group D antigen such as *E. pseudoavium*, *E. dispers*, *E. cecorum*, *E. sulfureus*, *E. columbae* and *E. saccharolyticus*. Presumptive identification of catalase negative Gram positive cocci (*Lactococcus*, *Leuconostoc*, *Pediococcus* and *Vagococcus*) and phenotypically similar genera and *Enterococcus* can accomplished by positive for BEA, PYR and LAP tests and growth in presence of 6.5% NaCl, 9.6 pH and temperatures at 10°C & 45°C. Confirmation of *Enterococcus species* requires still more biochemical tests for complete identification to the species level. Till date there are more than 29 distinct species included in the genus *Enterococcus*, but they lack many of the phenotypic characteristics typical of genus. Luckily, the majority of these species are not usually found in human clinical specimens.[12,13]

The *Enterococcus species* can divided in to five physiological groups (I, II, III, IV and V) based on acid formation from mannitol and sorbose and on hydrolysis of
arginine. The grouping by conventional phenotypic tests can be performed in any laboratory. Identification of enterococcal species by conventional tests is not rapid and
may require incubation of the tests for up to 10 days. However, identification also can make after 2 days of incubation.[12] Now days many automated system are available for rapid (4-6 hours) identification of enterococci.

**Summary of different Enterococcus species** [12,19]

**Group I**

*Enterococcus species* form acid from mannitol, sorbitol and sorbose containing broths, but do not hydrolyze arginine. It consists of *E. avium, E. pseudoavium, E. raffinosus, E. gilvus, E. saccharolytics, E. malodoratus, E. pallens* and *E. species CDC PNS E3*. They are differentiated by arabinose, raffinose, PYR, BEA tests and pigment production.

*Enterococcus avium*: Isolated from avian, canine and human gastrointestinal tracts; strains may carry both Lancefield group D and group Q carbohydrate antigen; this species produces H$_2$S gas. It has been isolated from cases of bacteremia and osteomyelitis.

*Enterococcus gilvus*: It was originally isolated from a bile specimen of a patient with cholecystitis. It produces pale yellow pigment and delayed growth at 45°C.[75]

*Enterococcus malodoratus*: Isolated from Gouda cheese and unpasteurised milk products; malodoratus means “ill smelling”. It also produces H$_2$S gas.

*Enterococcus pallens*: It was isolated from peritoneal dialysis specimen of a patient in whom peritonitis developed from perforated intestine. The bacterium produces bright yellow pigment. The strains are negative for PYR hydrolysis.[75]

*Enterococcus pseudoavium*: Genetic related studies & certain characteristics differentiate this species from *E. avium*; this was isolated from a case of bovine mastitis.

*Enterococcus raffinosus*: Originally considered to be related to *E. avium*; named for its ability to produce acid from raffinose; recovered from human infection, including blood culture, urine, abscesses and vertebral osteomyelitis.

*Enterococcus saccharolyticus*: Recovered from cows; it grows at 10°C to 45°C and in broth containing 6.5% NaCl, but does not react with group D antisera.

*Enterococcus species* CDC PNS-E3: CDC “proposed new species” isolated from brain tissue obtained from an 11 month old patient in Honolulu, Hawaii, 2001.[76]

**Group II**
Enterococcus species form acid from mannitol and hydrolyse the arginine but fail to form acid from sorbose. It comprises of *E. faecalis*, *E. faecium*, *E. mundtii*, *E.*
casseliflavus, E. gallinarum, E. haemoperoxidus, E. solitarius, E. seriolicida E. species and CDC PNS E2. All these species are separated by acid formation from arabinose, lactose, sorbitol, sorbose and yellow pigment production.

**Enterococcus faecalis:** Most frequent isolate from human clinical specimens and gastro-intestinal tract. It also found in intestinal tract of poultry, cattle, pigs, dogs, horses, sheep and goats.

**Enterococcus faecium:** It has found in human clinical specimens; generally more resistant to antimicrobial agents than *E. faecalis*; also found in gastrointestinal tracts of various species of animals.

**Enterococcus casseliflavus:** Recovered from plants, soil and rarely from faeces of chicken; earlier classified as subspecies of *E. faecium*, produces yellow coloured pigment and is motile. This organism is an opportunistic agent in human and has been isolated from patients with bacteraemia.

**Enterococcus gallinarum:** It was isolated from chicken faeces. It has also been isolated from an infection in a haemodialysis patient, as cause of native-valve endocarditis and from blood culture.

**Enterococcus mundtii:** Yellow pigmented, non haemolytic, non-motile organisms isolated from plant, soil and the gastrointestinal tracts of cattle, pig and horses; named after JO Mundt an American microbiologist. This species has been isolated from human thigh abscess, an operated sinus mucosal specimen and blood sample of patient having rheumatoid arthritis and chronic granulocytic leukaemia.

**Enterococcus haemoperoxidus:** It has been isolated from surface water, swimming pool and drinking water in north Moravia region of the Czech Republic. They can grow on blood free medium.

**Enterococcus species** CDC PNS-E2: CDC “proposed new species” isolated from blood culture from patient in Los Angeles in 1997.

**Group III**

Enterococci hydrolyze arginine but do not form acid from mannitol, sorbitol and sorbose. This group consists of *E. dispar, E. durans, E. hirae, E. ratti* and *E. villorum (porcinus).* Out of these species *E. hirae* and *E. dispar* can be easily identified by formation of acid from sucrose and raffinose, and growth at 45°C. However, other new species have very similar phenotypic profile; they can be differentiated by reactions in the litmus milk, hydrolysis of hippurate and acid formation from trehalose and xylose.
Uncommon mannitol negative variant strains of *E. faecalis* and *E. faecium* resemble
Review of literature [Bacteriology] 2015

species in this group. However, *E. faecalis* strains are positive for pyruvate test but not for acid formation from arabinose, raffinose or sucrose and *E. faecium* variants form acid from arabinose.

**Enterococcus dispar:** This species was originally thought to be a biochemical variant of *E. hirae* but analysis of 16S rRNA indicated that this organism is indeed a less described species; it does not grow at 45°C unlike other enterococci and recently, recovered from human specimens,\(^79\) including stool and synovial fluid.

**Enterococcus durans:** This species is found mainly in the milk and other dairy products but a rare clinical isolate. *E. durans* was isolated as a causative agent of native-valve endocarditis.

**Enterococcus hirae:** It was isolated from chicken crops and hog’s faeces\(^57\) and gastrointestinal tracts of cattle, pigs, dogs, horses, sheep, goats and rabbits. It causes growth dispersion in chickens. *Enterococcus hirae* ATCC 8043 has complex nutritional requirements and is used in food industry as a bioassay organism for amino acids and vitamins. *E. hirae* was isolated from the blood of a 49 years old patient with end stage renal disease who was undergoing haemodialysis.\(^57\)

**Enterococcus villorum:** Isolated from gastrointestinal tracts of dogs; phenotypically identical with *E. porcinus*, this is considered as a junior synonym of *E. villorum* and originally isolated from faeces of pigs with diarrhoea.\(^80\)

**Enterococcus ratti:** This new species was originally isolated from the intestine and faeces of rats with diarrhoea.

**Group IV**

Enterococci do not form acid from mannitol and sorbose and fail to hydrolyse arginine. It is composed of four species *E. asini*, *E. phoeniculicola*, *E. cecorum*, *E. sulfureus* and *E. species CDC PNS E1*. All these species can be differentiated by acid formation from arabinose, raffinose, and sorbitol and pigment production ability.

**Enterococcus asini:** It was found in the donkey and it closely related to *E. avium*, *E. pseudoavium* and *E. faecium*.

**Enterococcus cecorum:** Newly reclassified enterococcal species found in intestine of chicken. It differs markedly from all enterococcal species, in failing to grow at 10°C, in 6.5% NaCl broth. It lacks group D antigen and is PYR negative \(^{48}\) and it requires CO\(_2\)
for its growth. It has been isolated as a cause of peritoneal dialysis associated peritonitis, recurrent bacteraemia peritonitis and spontaneous peritonitis with empyema and has been isolated from blood culture of a patient with severe malnutrition.
**Enterococcus sulfureus:** Newly described yellow-pigmented species; recovered from plants; has not yet been isolated from humans.

**Enterococcus phoeniculicola:** This species is found in preen glands of wild red-billed Wood hoopoes; does not grow on BE agar or in 6.5% NaCl broth.

**Enterococcus species CDC PNS-E1:** CDC “proposed new species” isolated from the blood of patient in Evanston, Illinois, in 1991.\(^{76}\)

**Group V**

Enterococci that fail to hydrolyze arginine and form acid from sorbose but produce acid from mannitol. However, these species can be differentiated by acid formation from sorbitol and raffinose and hippurate hydrolysis. This group contains *E. columbae, E. canis* and *E. moraviensis.*

**Enterococcus columbae:** Newly reclassified streptococcal species found in intestinal tracts of pigeon, it is closely related to *E. cecorum* and *E. avium.* It is characterised by being PYR- negative and unable to grow in 6.5% NaCl broth.

**Enterococcus canis:** It was originally isolated from a dog with chronic otitis externa; probably a resident of the canine gut. It grows in 6.5% NaCl broth and is PYR- positive.

**Enterococcus moraviensis:** New environmental species, isolated from drinking water in North Moravia region of Czech Republic.\(^{51}\)

There are several commercially available miniaturized, manual, semi automated and automated identification systems for identification of *Enterococcus species.* Since, their introduction, these systems have been updated to improve their performance characteristics and expand their identification capabilities, as investigators are more aware of their inaccuracies. In general, these systems are reliable for identification of *E. faecalis,* and to lesser extent *E. faecium.* Commercial systems now available for identification of *Enterococcus species* include: the APE 20S and the API Rapid ID32 STREP systems (BioMeerieux, USA), the crystal Gram-positive and the crystal rapid Gram-Positive identification systems (Becton Dickinson Microbiology systems), the Gram-Positive Identification Card of the Vitek system (BioMeerieux) and the Gram-Positive Identification panel of the MicroScan Walk/Away system (Dade MicroScan, USA). In general, approximately 80 percent of all enterococcal isolates can be accurately identified by any one of these system. The reports of unusual or uncommon *Enterococcus species* isolated from human infections have been reported from time to time in the literature. In the era of compromised host, the clinical microbiologist should...
be aware of these other species and methods for their identification. Identification of unusual species by commercial system should confirmed by a reference method before being reported.[12,13]

The molecular methods based on the analysis of different target molecule present in the enterococcal cells, such as DNA-DNA hybridization and sequencing of the 16S rRNA gene, have been primarily used for taxonomic purpose in special laboratories. In the past decade however, the applications of molecular techniques for the identification of Enterococcus species has expanded dramatically. The range of molecular procedures proposed for identification of Enterococcus species include: analysis of whole cell protein (WCP) profile by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE); vibrational spectroscopic analysis; proton magnetic resonance spectroscopic analysis; randomly amplified polymorphic DNA (Rapid) analysis; sequencing analysis of the 16S rRNA gene; fragment-length polymorphism analysis of the amplified 16S rDNA gene; broad-range amplification (BR-PCR) of 16S rDNA; sequencing of domain V of the 23S rRNA gene; amplification of the rRNA or the tRNA intergenic spacers or of the D-Ala: D-Ala ligase (ddl) and the vancomycin resistance (van) genes; sequencing of the ddl genes or of the manganese-dependent superoxide dismutase (sodA<sub>int</sub>) gene; sequencing and hybridization of the chaperonin (cpn60) gene; amplification and probing of the enterococcus protein A (efaA) genes or of the E. faecalis adhesin for collagen (ace) gene; and amplification of elongation factor EF-Tu (tuf) gene, or the pEM1225 gene.[12]

Among the molecular techniques proposed to identify the enterococcal species, SDS-PAGE analysis of WCP profile is the only one that has been extensively evaluated and recommended for general use. This method has shown to be reliable for the differentiation of typical and atypical Enterococcus strains, since WCP profiles are species-specific. Analysis of WCP using a standardized technique has revealed correlation between WCP profiles, DNA homology studies and the phenotypic tests. Sequencing the 16S RNA gene has been performed on all species of enterococci and the sequences are available via Gene Bank for the comparison purpose. Among other methods only few have performed, some do not appear to be practical for routine use in the clinical laboratories. While some of these recently developed molecular procedures, deserve consideration for expanding testing and future improvement.[12]
PATHOGENESIS AND VIRULENCE FACTORS

The enterococci have been for long considered innocuous organisms, existing as normal commensals in the gastrointestinal tract. Certain predisposing conditions allow this organism to invade extra intestinal regions and cause infections. Surprisingly little is known about the factors that contribute to the ability of enterococci to cause infections in humans. To act as pathogens, enterococci must first adhere to host tissues. During the process of tissue invasion, enterococci encounter an environment vastly different from those at sites of colonization, with higher redox potentials, limited essential nutrients, phagocytic leukocytes and other host defences. Enterococci express genes favouring growth under these alternate environmental conditions. As they have become increasingly apparent in recent years, enterococci express factors that permit adherence to host cells and extracellular matrix, facilitate tissue invasion, effect immuno-modulation and cause toxin-mediated damage.

The ability of the organism to acquire newer traits makes it more virulent enabling it to colonise in newer areas in the host and cause infection. There is no single factor, which is responsible for the virulence of the organism. Enterococci have the ability to modulate virulence by selective high-frequency deletion of specific regions of DNA. The one-third of Pathogenicity Island appeared to have evolved from integration into chromosome of a conjugative plasmid sequence.

The enterococci are important, often multi-drug-resistant pathogen, that are third leading cause of endocarditis and third most common cause of nosocomial bacteremia. The virulence mechanisms used by enterococci have not been well understood. There are many postulates regarding virulence of enterococci. They have virulence factors such as cytolysin, aggregation substance, extracellular surface protein, extracellular superoxide, lipoteichoic acid and coccolysin, serine protease, capsule, cell wall polysaccharide. These virulence factors and other metabolic and multidrug resistance capabilities may allow enterococci to proliferate as nosocomial pathogens. Most of infections are nosocomial and include urinary tract infections, bacteremia, endocarditis,
mixed infection of abdomen and pelvis, wounds and occasionally ocular infection. CNS and respiratory tract infections are rare.[25] Enterococci have attracted much attention in recent times due to their increased recognition as cause of nosocomial infection, ‘super infection’ in patients who receives antibiotics.[21]
**E. faecalis** strains isolated from different sources possess distinctive patterns of potential factors, with a large numbers of genes that encode potential virulence factors among isolate from UTIs.\(^{[82]}\) The prevalence of virulence factors is more common among the isolates from clinical specimens than sewage samples. Presence of virulence factors in isolates from sewage samples suggests that can be potentially harmful for human. Extracellular surface protein and hyaluronidase are only present in isolates from clinical specimens but not from sewage samples suggesting the loss of the virulence genes in the sewage milieu.\(^{[83]}\) Charles Franz *et al.* showed that virulence factors such as haemolysin, aggregation substance, Gelatinase and extracellular surface protein also occur among food enterococcal isolates.\(^{[52]}\)

Enterococci colonize the intestine and interact in complex and largely unstudied associations with other bacteria, the intestinal epithelium and other components of the mucosal interface. Comparatively little is known about the nature of enterococcal colonization of the human gastrointestinal tract except that enterococci occur in relative abundance in human faeces.\(^{[25]}\)

The virulence of an organism is regulated by virulence coding genes present on the genome in special regions, which are known as pathogenicity islands (PAI). The PAI of enterococci was first identified in the genome of multidrug resistant strain of *E. faecalis* that had caused an outbreak of nosocomial infection in the 1980’s. The size of the gene is around 150 kb and encodes for 129 open reading frames (ORF). G+C content has found to be 32.2%. There are some genes, which encode transposons, transcriptional regulators and proteins with known potential roles in virulence. The virulence traits encoded within *E. faecalis* PAI are cell surface associated protein namely enterococcal surface protein [ESP], the secreted toxin cytolysin and aggregation substance.\(^{[21]}\)

**Enterococcal Genetic Exchange:**

Plasmid and transposons encoded genes provide survival advantages to the organisms in unusual environments. Such traits include antibiotic or heavy metal resistance, bacteriocin activity, metabolism of unusual substrates and virulence factors. Many putative enterococcal virulence factors reside on conjugative plasmids. These plasmids spread horizontally between strains in a natural environment like gastrointestinal tract.\(^{[20]}\)
Enterococci possess potent and unique abilities to exchange genetic material among themselves and also with other genera. At least three conjugative systems exist by which enterococci naturally transfer genetic elements. First, narrow-host range, pheromone-responsive plasmids transfer at high frequencies ($10^3$ to $10^2$ per donor). Second, many plasmids with a broad host range (e.g. pAMB1 readily transfer at low frequency ($<10^{-6}$ per donor) among enterococci, Streptococcus species, Staph. aureus, Lactobacillus species, Bacillus subtilis and other species. Transfer requires contact between donor and recipient cells can occur in vitro (on solid surfaces i.e. filters membrane and/or in broth) and in vivo.

Those systems, which transfer readily in broth, make use of sex pheromones to generate cell-to-cell contact, whereas those that transfer poorly do not (Brown and Clewell, unpublished data) have sex pheromones. Recipient strains excrete soluble, protease-sensitive, heat-stable substances which induce certain donor cells to become adherent. This induction facilitates the formation of donor-recipient mating aggregates that arise from random collisions. Since a cell-free filtrate of a recipient also elicits an aggregation (clumping-clumping) response when it mixed with donors, this substance has referred to as clumping-inducing agent (CIA). Interestingly, donors harbouring different conjugative plasmids respond to different CIAs. A given recipient actually produces multiple pheromones. The acquisition of a given plasmid shuts off production of only the related pheromone, whereas the cell continues to produce other pheromones, which can induce other donors with different conjugative plasmids.

Donor

Recipient

Expression of sex pheromone by a recipient E. faecalis strain and response by a donor containing a pheromone-sensitive conjugative plasmid.

The isogenic donor-donor mating also may occur by using two distinguishable transposons (Tn916) and (Tn917). When only one of the donors induces, transfer occurred only in the direction from the induced strain to the un-induced strain. If both donors induce aggregation, then once the cells make contact, transfer occurs equally, well in both directions, regardless of which donor has induced with cADI before mating.
Activity of Virulence Factors [20]

<table>
<thead>
<tr>
<th>Factor</th>
<th>Observed activities and model systems used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytolysin</td>
<td>Lytic towards Gram-positive bacteria and selected eukaryotic cells; decreased lethal dose and time to death in murine peritoneal infection; destruction of retinal tissue in rabbit endophthalmitis; in combination with aggregation substance, increased mortality in substance, increased mortality in rabbit endocarditis.</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Facilitates binding of donor to recipient cells in pheromone mating response; augmented adherence to cultured renal tubular cells through Arg-Gly-Asp motifs; increased vegetation weight in rabbit endocarditis; in combination with cytolysin, increased mortality in rabbit endocarditis; invasion of enterocytes enhanced.</td>
</tr>
<tr>
<td>substance</td>
<td></td>
</tr>
<tr>
<td>Pheromones</td>
<td>Chemotactrant for neutrophils in vitro.</td>
</tr>
<tr>
<td>Lipoteichoic</td>
<td>Stimulation of cytokine production in cultured human monocytes; binding ligand for aggregation substance in pheromone mating response.</td>
</tr>
<tr>
<td>acid</td>
<td></td>
</tr>
<tr>
<td>Protease</td>
<td>Zinc-endopeptidase; ND*.</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>Mucopolysaccharidase; ND*.</td>
</tr>
<tr>
<td>AS-48</td>
<td>Bacteriocin with activity against Gram-positive and Gram-negative bacteria; ND*.</td>
</tr>
</tbody>
</table>

*ND—Not detected.

Comparison of virulence factors in *E. faecalis* and *E. faecium*[20,21,27]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Factor</th>
<th><em>E. faecalis</em></th>
<th><em>E. faecium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Antibiotic resistance</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Cytolysin</td>
<td>++</td>
<td>±</td>
</tr>
<tr>
<td>3</td>
<td>Aggregation substance</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Gelatinase</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Extracellular superoxide</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Extracellular surface protein</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Lipoteichoic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Coccolycin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Capsular polysaccharide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>MSCRAMM Ace /Acm</td>
<td>Ace +</td>
<td>Acm +</td>
</tr>
<tr>
<td>11</td>
<td>Pheromones</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Hyaluronidase</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>AS-48</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

++ Most; + some; ± rare; - none.

Pheromone activities can be quantified by using a simple micro-titre plate system; serial twofold dilutions are used, and the highest dilution of filtrate that still induces clumping of the appropriate responder (donor) cells has taken to represent the pheromone titre. The titre for a given filtrate varies somewhat with the particular responder system; this depends on the conjugative plasmid, as well as the host;
typically, titres range from 04 to 64. The pheromone production by recipient cells is
closely parallel with growth of cell. The CIA activity in filtrates gets off as cells enter the stationary phase.\textsuperscript{[85]}

Conjugative gene exchange occurs through usually transposons which found in Gram-positive as well as Gram-negative bacteria. One transposon has shown to transfer at low frequency from \textit{E. faecalis} to \textit{Listeria monocytogenes} in the intestinal tracts of gnotobiotic mice. Tn916 is the most thoroughly characterized conjugative transposon and is used extensively in molecular genetic studies. In addition gene exchange naturally may occur in enterococci through infection with bacteriophages. Enterococcal bacteriophages have been isolated from stool and have a narrow host range rendering them of potential use for strain typing. The ability of enterococcal bacteriophages to spread virulence traits or antibiotic resistance determinants is largely unknown.\textsuperscript{[20]}

Virulence factors among enterococci appear to be the cause of emergence of pathogenic lineages of other species. The emergence of genetic lineages with enhanced virulence results from the acquisition of new traits by genetic exchange. Among enterococci, traits that have been acquired by some lineages, have permeated the species to various degrees, and are suspected to relate to an enhanced ability to cause disease include antibiotic resistance determinants, a cytolytic toxin, gelatinase, aggregation substance, extracellular superoxide production, and enterococcal surface protein. All of these appear to fulfil the formal definition of virulence factor, in that they enhance the ability of the organism to cause disease. Autolysins have been best characterized in \textit{E. hirae} than \textit{E. faecalis}. The cell wall carbohydrates are still under study and there is little agreement about these carbohydrates as virulence factors. Other phenotypic and potential virulence factors that require further investigation include hyaluronidase, lipoteichoic acids, fibronectin and surface carbohydrates.\textsuperscript{[25]}

Enterococcal virulence is related to (A) adherence to host tissues, (B) modulation of host immunity, (C) toxic products and (D) Others (Extracellular Superoxide, Extracellular Surface Protein, Coccocolysin, MSCRAMM ACE, biofilm formation and antibiotic resistance determinants)

\textbf{A. ADHERENCE TO HOST TISSUES}

Bacterial adherence to host tissues is an essential primary stage in the infection
progression. For gastrointestinal commensals such as enterococci, adhesins that promote
binding to eukaryotic receptors on mucosal surfaces play a critical role in maintenance of colonization. Unless specific attachment of enterococci they suppose to go away with mass flow of intestinal contents. Adherence through surface-exposed adhesins to epithelial cells, endothelial cells, leukocytes or extracellular matrix is an earliest stage in infection.\textsuperscript{[20,45]}

1. Aggregation Substance:

Aggregation substance (AS) is a surface-bound, plasmid encoded a protein that promotes clumping of the organisms to facilitate the plasmid exchange.\textsuperscript{[19]} The sex pheromone system of \textit{Enterococcus faecalis} was first described in 1978. Sex pheromones are small hydrophobic peptides consisting of seven or eight amino acids.\textsuperscript{[45]} It is a pheromone-inducible\textsuperscript{[20]} surface protein of \textit{E. faecalis}, which encourages mating aggregate formation during bacterial conjugation.\textsuperscript{[18]} The adhesin is proteinaceous in nature and is located on the surface of producing cells; appear as a hair like structure. The adhesion of donor and recipient cells leads to clumping reaction,\textsuperscript{[52]} easily visible to naked eyes and enable the conjugative transfer of the so-called sex pheromone plasmids. Transfer of the plasmid from donor to donor does not occur. Nucleotide (DNA) sequencing of the structural gene, \textit{asal}, for the aggregation substance revealed the presence of two amino acid motif Arg-Gly-Asp-Ser, it may mediate binding to eukaryotic (renal tubular) cells via a class of eukaryotic receptors, the integrins [Photograph D].\textsuperscript{[20,45]}

The pheromone-induced donor cells have a new antigen on their surfaces. The surface material is referred to as aggregation substance and since it is sensitive to trypsin and pronase, this aggregation substance appears to be proteinaceous. When preparations were subjected to specific immunological staining procedures involving conjugated horseradish peroxidase and then analyzed by electron microscopy, an amorphous surface material (presumably aggregation substance) was observed on the surfaces of induced cells but not on uninduced cells. Pilus-like structures were not observed; however, the possibility that small microfimbriae that are difficult to resolve, coat the surface remains. In the absence of immunological strains, no surface differences between induced and uninduced cells could be detected by either transmission or scanning electron microscopy\textsuperscript{[85]} as shown in photograph A, B and C.
**Photograph- A.**
The uninduced enterococcal cells do not show aggregation substance.

**Photograph- B.**
The induced enterococcal cells showing aggregation substance.

* by Yoshihiko Yagi

**Photograph- C:**
Immunogold - labelled aggregation substance.

Anti - Asc10 monoclonal antibody has been used to label aggregation substance expressed on the surface of cells. Antibody specifically bound to aggregation substance was labelled with 12 nm colloidal gold - tagged.

**Photograph D.**
Electron Microscopic demonstration of preferential binding of *E. faecalis* via hair like structures to the microvilli of renal tubular cells. (Bar 1 µm)
Aggregation substance appears to be the anchor in the bacterial membrane; by its C terminus in a manner common to cell wall-associated proteins of Gram-positive bacteria. The deposition pattern of aggregation substance encoded by the pheromone-responsive plasmid pCF10, when cloned and constitutively expressed from the construction pINY1801, is shown at high resolution in photograph: C. In addition to facilitating exchange of plasmids carrying virulence traits and antibiotic resistance genes, aggregation substance may augment enterococcal adherence to intestinal and renal epithelial cells and cardiac vegetations.[20]

In vitro, aggregation substance mediates adhesion to a variety of eukaryotic cell surfaces, such as cultured pig renal tubular cells and promotes internalization by cultured human intestinal cells.[17,26,87] Aggregation substance was also studied in the rabbit model of E. faecalis endocarditis[19,25] and found to be associated with greater and early establishment[21] of the vegetation size compared to vegetations caused by isogenic aggregation substance-defective strains, although these infections were not observed to be lethal. More recent studies suggest that aggregation substance and its cognate ligand, binding substance, may lead to destruction of myocardial and pulmonary tissues.[25]

Aggregation substance also encourages direct opsonin-independent binding of E. faecalis to human neutrophils via complement receptor type 3. In addition, other receptors on the neutrophils surface appear to promote intracellular survival of E. faecalis inside neutrophils.[19,25,86] This type of AS mediated intracellular survival was not only limited to neutrophils but also found with other phagocytes, such as monocyte derived macrophages and cell from the murine macrophages.[86] In a comparison of ingested, non-opsonised aggregation substance-bearing E. faecalis, to ingested, opsonised E. faecalis, response for activation of neutrophils is associated with rapid alteration in the array of surface proteins, there were higher levels of phagosomal pH, extracellular superoxide, phagosomal oxidant production, surface Mac-1 expression and shedding of L-selectin.[25,86] Vanek N.N. et al. found that complement receptor type 3 (CR3) on the surface of neutrophils plays an important role in AS-mediated bacterial adhesion. Approximately 85% inhibition of adhesion was noted when neutrophils were treated with monoclonal antibodies directed against either subunit of CR3, along with similar diminution in binding to leucocytes adhesion deficiency (LAD), neutrophils expressing only low level of CR3.[17] The low level bacterial binding to neutrophils was seen with strains lacking AS, in comparison to strains with AS. The AS containing E.
faecalis has been frequently isolated from clinical specimens; but less frequently isolated from stool of healthy volunteers.\(^{[17,86]}\) The enterococci, even those lacking AS, appear to bind neutrophils in low numbers via an alternate mechanism that does not involve CR3 or potentially linked receptors. This is clear by the incapability to completely block binding of AS bearing E. faecalis to neutrophils. In addition, the residual levels of bacterial binding seen after blockage of CR3 or with LAD PMNs are similar to E. faecalis lacking AS. The presence of multiple adhesins on an individual pathogen is common.\(^{[17]}\)

In vivo, aggregation substance may contribute to the pathogenesis of enterococcal infection through a number of mechanisms. Aggregation substance is known to be induced by pheromone signals and by serum, suggesting that aggregation substance-expressing cells likely form larger aggregates in vivo than cells not expressing this trait. Aggregation contributes to bacterial virulence in other systems and it is presently unknown how the simple act of aggregation may influence phagocytosis and the subsequent fate of the organism.\(^{[25]}\) The enterococci expressing AS are found to resist phagocytosis significantly more than isogenic AS negative strains by inhibition of respiratory burst (production of reactive oxygen) in the macrophage.\(^{[21]}\)

There are also indications that aggregation substance may bind and present its cognate ligand, which is believed to be related to teichoic acids, on the surface of the organism, possibly resulting in super antigen activity\(^{[25]}\) and which, stimulate tumour necrosis factor production by macrophage and lymphocytes, which could subsequently increase the amount of CR3 on the surface neutrophils. This could provide a route for greater AS-mediated bacterial binding to neutrophils.\(^{[17]}\) Finally, aggregation substance increases the hydrophobicity of the enterococcal surface, which may induce localization of cholesterol to phagosomes and prevent or delay fusion with lysosomal vesicles. The aggregation also enhances the organism’s ability to attach with intestinal epithelial cells. As most cytolytic strains of E. faecalis also express aggregation substance, which work synergistically, as shown by the results of studies in endocarditis. An aminoglycoside-resistant lineage of E. faecalis expressing cytolysin and aggregation substance proved particularly virulent. The phenotype VanB strain, possesses a naturally, insertionally disrupted cytolysin operon (unpublished observations) may also contribute to the limited spread of this isolate.\(^{[25]}\) Thus, AS may play an important role in the pathogenesis of E. faecalis infections.
2. **Capsular Polysaccharide and Cell Wall Carbohydrate:**

Guzmán *et al.* first reported carbohydrate adhesins in enterococci in 1989. This group observed that *E. faecalis* strains isolated from urinary tract infections adhered efficiently to urinary tract epithelial cells in vitro. In contrast, *E. faecalis* strains isolated from the blood of patients with endocarditis adhered efficiently to the Girardi heart human cell line. When urinary tract isolates were grown in the presence of serum, adherence to the heart cell line had enhanced.[20]

A capsular polysaccharide present on the surface of both *E. faecalis* and *E. faecium* were purified and chemically characterised. The protective antibodies are produced against purified carbohydrate and suggesting that these antibodies may be useful for prevention of enterococcal infections. The glucose, galactose and glycerol phosphate are present in purified cell wall carbohydrate.[21]

3. **Other Adherence Mechanisms**

Other factors also promote the adhesion of enterococci to host tissues but very limited information is available about them. Shorrock and Lambert investigated the binding of fibronectin and albumin by *E. faecalis*. They observed that fibronectin bind *E. faecalis* with higher affinity than albumin binding. Treatment of *E. faecalis* with protease reduced binding, suggesting a surface receptor comprising protein and/or carbohydrate components.[20]

![Photograph E: Adhesions of *E. faecium* to Vero cell line.](image)

Photograph E: Adhesions of *E. faecium* to Vero cell line.

*a*- Negative control, *b*- Adhesions (magnification X 1000)

**B. MODULATION OF HOST IMMUNITY**

The pathogens breach mucosal or skin barriers and adhere to host tissues or cells
and infection can develop only if other defences are neutralized, avoided or restricted.
Professional phagocytes such as neutrophils, monocyte and macrophages provide nonspecific, but powerful, host defences against all types of pathogens. Neutrophils, in particular, migrate efficiently to sites of infection in response to chemotactic signals, use complement and antibody for pathogen recognition and kill the ingested organisms by oxidative and non-oxidative mechanisms.\cite{20}

1. Lipoteichoic Acids:

Membrane-associated lipoteichoic acids common among prokaryotic organisms are amphipathic polymers composed of a hydrophilic polyglycerol phosphate backbone linked via an ester bind to a hydrophobic glycolipid tail. Within single organism, lipoteichoic acid exists in varying forms especially it varies in its glycerophosphate chains.\cite{13} For enterococci these surface molecules have shown to be identical to the group D antigen. Enterococcal lipoteichoic acid reversibly binds to human erythrocytes.\cite{20} It may induces production of tumour necrosis factors (TNF) and interferon.\cite{19,20}

The lipoteichoic acids from \textit{S. aureus} and \textit{S. pneumoniae} failed to induce monokine production but enterococcal lipoteichoic acids at 0.5 to 5.0 µg/ml concentrations induced release of all three monokines. The levels of monokines stimulated by enterococcal lipoteichoic acid were similar to those observed after exposure to Gram-negative lipopolysaccharides. Purified lipoteichoic acid from \textit{E. faecalis} inhibited pheromone-induced aggregation of bacterial cells. It acted as the binding substance recognized by aggregation substance on donor cells. The enterococcal lipoteichoic acid may serve as a virulence factor by modulating inflammatory responses.\cite{20}

2. Complement and Neutrophils:

Neutrophils kill enterococci in the presence of complement and specific rabbit anti-enterococcal immune globulin by both opsonisation and alternative complement pathway.\cite{87,89} Strains capable of expressing protease, cytolysin or aggregation substance proved no more resistant to phagocytosis than strains lacking these phenotypes.\cite{20}

The enterococci are opsonised, is consistent with their apparent lack of capsular
polysaccharide. However, pathogens phagocytised by neutrophils rarely survive
exposure to hypohalous acids resulting from a respiratory burst. Macrophages are less efficient in killing ingested pathogens than neutrophils, because they lack myeloperoxidase and cannot produce hypohalous acids from reactive oxygen species. Although enterococci are catalase negative, they express a flavin containing NADH peroxidase to degrade hydrogen peroxide. Enterococci also possess an oxygen-inducible superoxide dismutase to catalyze conversion of superoxide to hydrogen peroxide. Whether these enzymes enhance survival of enterococci after phagocytosis by macrophages awaits investigation.[20]

*E. faecium* isolates were more resistant to PMN-mediated killing than the *E. faecalis* isolates, and their killing was not enhanced by pre-opsonisation with homologous serum containing both active complement and anti-enterococcal antibodies. Different phenotypic traits of *E. faecalis*, including antimicrobial susceptibility and production of gelatinase, haemolysin or aggregative substance did not influence the susceptibility to killing by neutrophils. Nevertheless, factors associated with complement activation, binding to PMNs, or intra-phagosomal microbicidal systems may be responsible for this species specific resistance, and further studies exploring these speculations are being pursued.[88]

### 3. Pheromones:

These are small peptides of 7-8 amino acids which are secreted by the organism and that promote the conjugative transfer of plasmid DNA between strains.[19,20] It also appears that these same molecules may act as chemoattractants for neutrophils, thereby helping to augment the inflammatory response to infection.[19] These peptides are chromosomally encoded and are called as pheromones. *E. faecalis* simultaneously secreted multiple pheromones. In addition to pheromones, would probably be a prime target for R-plasmids,[85] each pheromone-responsive plasmid encodes a secreted peptide that acts as a competitive inhibitor of its corresponding pheromone.[20] Interestingly, the ability to respond to and to produce clumping-inducing agent activities was significantly more frequent among strains resistant to one or more drugs than among drug-sensitive strains. Thus, pheromones may contribute to the evolution of drug resistance in *E. faecalis*. Some of the pheromones produced and released by *E. faecalis* promote a chemotactic cell response and activate the respiratory burst in human neutrophils.[88]
The fact that a single recipient strain of *E. faecalis* may produce numerous sex pheromones specific for different donors seems surprising at first, since it is possible that such cells have never before encountered the related plasmids. The pheromones may have other functions in the recipient cells or may represent degradation products of larger proteins. Plasmids may have evolved in such a way as to take advantage of such molecules as mating signals to facilitate their dissemination.[85]

C. TOXIC PRODUCTS

1. **Cytolysin or Haemolysin:**

   Enterococcal haemolysin is a cytotoxin. *E. faecalis* (up to 60%) produces a cytolysin, which lyses a broad range of eukaryotic and prokaryotic cells.[87] Diagnostically, this toxin causes a β-haemolytic reaction on human, rabbit, equine, bovine[19] and horse[21] blood agar (but does not haemolyse sheep blood agar, which is frequently used in clinical microbiology laboratories). In addition to toxin activities, the cytolysin of *E. faecalis* possesses bacteriocin activity against a broad range of Gram-positive bacteria, including non-cytolytic enterococci.[18,25] The self-protective mechanism of immunity for the cytolysin-producing strain has recently been described. A number of independent studies using different model systems have consistently observed a role of *E. faecalis* cytolysin in the toxicity of enterococcal infection.[25]

   The haemolysin producing strains of enterococci have shown to be virulent in animal models[87] and human infections.[21] The rabbit endophthalmitis,[90] endocarditis model[19] and murine peritonitis[18] was used to examine in detail the contribution of cytolysin to virulence, by using isogenic strains of *E. faecalis* differing only in this trait. The *E. faecalis* cytolysin contributes to the occurrence and severity of endophthalmitis.[90] The *E. faecalis* strain produced the cytolytic toxin, the combined beneficial effect of antimicrobial and anti-inflammatory therapy would be completely offset by the organo-toxic activity of the cytolysin, which destroyed the organ even though all other aspects of the infection were successfully managed. These studies show that even in an organ with limited immune response, enterococcal disease has an important inflammatory component as well as an organo-toxic component if the offending organism is a cytolysin producer.[25]

   Lethality is significant increase due to cytolytic enterococci as compared to non-cytolytic enterococci. Vegetations due to cytolytic enterococci exhibited a significant
increase as compared to non-cytolytic enterococci. Some studies represent important refinements on the original acute lethality observations made in the murine intraperitoneal challenge model. The number of organisms used in this challenge was very large (10^5 to > 10^{10} cfu). The cytolysin was found to significantly enhance the lethality.[25]

The haemolysin-bacteriocin contributes to virulence in experimental and human enterococcal infections; as well as they produce larger than normal zones of haemolysis on blood agar rendered the strain are more virulent. In rabbit endophthalmitis model, infection caused by cytolytic *E. faecalis* resulted in 99% loss of retinal function compared with loss of only 74% infection caused by non-haemolytic strains. Microscopy showed that near-total destruction of retina (disorganisation) with cytolytic *E. faecalis* was seen, while non-cytolytic strains produced only few changes but structurally intact.[18,90] A study in Japan found haemolytic strains in 60% of isolates from adults with systemic infection due to *E. faecalis* in contrast to only 17% of faecal isolates from healthy adults colonized with *E. faecalis*.[18]

Haemolysin production can be detected by inoculating enterococci on freshly prepared beef-heart infusion agar supplemented with five percent horse blood. Plates are incubated overnight at 37°C in a carbon dioxide chamber and evaluated after 24 or 48 hours. A clear zone around the colonies on horse blood agar is considered as positive. Expression of haemolysin or cytolysin is regulated by a novel, two-component regulatory system via quorum sensing mechanism.[21]

Cytolysin genes occur as chromosomal elements. The most extensively characterized cytolysin-encoding plasmid[87] is PAD1. The various investigators termed the two operationally defined components, L for lysin and A for activator based on the kinetics of interaction. Transposon insertional mutation localized the cytolysin determinant on PAD1 to 8 kb of DNA. Reading frames cylL1, cylL2, cylM and cylB are relevant to expression of component L, whereas cylA is the only reading frame necessary for expression of component A. About 20% of humans are normally colonizing with cytolytic Enterococci. In one study, cytolytic strains determined by regression analysis to be associated with a fivefold increased risk for death within 3 weeks of bacteremia compared with patients with bacteremia caused by non-cytolytic strains.[20]
2. **Gelatinase (protease):**

Gelatinase is a protease, produced by enterococci which are capable of hydrolyzing gelatin, collagen, casein, haemoglobin and other peptides. Gelatinase producing strains of *E. faecalis* have shown to contribute to virulence of endocarditis in an animal model. Gelatinase production in the laboratory can be detected by inoculating the enterococci on freshly-prepared peptone-yeast extract agar containing gelatine plates incubated at 37°C overnight and cooled to ambient temperature for two hours, show a turbid halo or zone around the colonies if it is positive for gelatinase production. *E. faecalis* isolated from endocarditis (100%), blood (64%) and stool (53%) showed positive for gelatinase production.[21] Isogenic strains of *E. faecalis* differing in gelatinase production appear in **acute toxicity** in the bolus LD 50 murine model.

Suet *et al.* reported the sequence of protease gene (gel E). The same workers used semisolid media supplemented with 3% gelatin or 1.5% skimmed milk media to demonstrate protease production. Gold *et al.* detected the potential contribution of enterococcal protease to virulence in 1975. Kunhen *et al.* reported that 63.7% of *E. faecalis* from intensive care units produce protease.[20]

3. **Hyaluronidase:**

Hyaluronidase is a cell surface associated enzyme, which cleaves the mucopolysaccharide moiety of connective tissue or cartilage. It has implicated to act as spreading factor for dissemination of microorganisms. Detection of hyaluronidase production by microorganisms is accomplished by inoculation into semisolid media containing hyaluronic acid. Hyaluronidase in other microorganisms has provided an indirect basis for speculating that this enzyme contributes to enterococcal virulence. Enterococcal hyaluronidase could play a role in invasive diseases.[20]

4. **AS-48:**

The basic peptide is lytic via the generation of pores in cytoplasmic membrane of target cells that leads to depolarization. It also induces lysis of selected enterococci by activation of an autolysin. AS with the cytolysin operon, AS-48 has found to encode by a transmissible plasmid. Prevalence of AS-48 producing strains among human commensals and infections isolates is yet to be defined.[20]
D. OTHER VIRULENCE FACTORS

1. Extracellular Superoxide:

Extracellular superoxide production trait is associated with enterococcal virulence in bacteremia and appears to vary among isolates. Most *E. faecalis* and some *E. faecium* strains generate substantial extracellular superoxide, with significantly greater production by invasive strains than by commensals isolates. Superoxide production is observed to enhance in vivo survival of *E. faecalis* especially in mixed infection with *Bacteroides fragilis*.\[21,25\]

2. Extracellular Surface Protein (Esp):

Enterococcal surface protein is a cell wall-associated protein in *E. faecalis* isolates and appears to be associated with enterococcal virulence. Extracellular surface protein was initially derived from the original *vanB E. faecalis* clinical isolate. The *esp* gene encodes a large bacterial surface protein with an interesting structure.\[87\] It is currently hypothesized that it has the central repeat region which serves as a retractable arm, \[19,25\] extending the N-terminal globular domain through the cell wall to the surface. The numbers of central repeats are found to vary between 3 and 11 in various *E. faecalis* isolates, supporting this hypothesis.

A statistically significant (P<0.001) association was noted with infection derived *E. faecalis* isolates compared to isolates from healthy individuals.\[87\] ESP enhance the persistence of *E. faecalis* in urinary bladder during experimental urinary tract infections. This shows that the Esp may help the organism to adhere to bladder epithelium through specific components of the bladder wall such as mucin or uroplakin.\[21\] There is association of *esp* with increased virulence, colonisation & persistence in urinary tract and biofilm formation.\[83,91\] Some times, in immune deficiency conditions it may facilitate immune evasion that protects the organism from antibodies. PCR amplification detected *esp* gene in only 3% of *E. faecalis* stool isolates, but 41% of *E. faecalis* blood isolates and 42% of *E. faecalis* endocarditis isolates.\[19,25\] The gene was not detected in isolates of *E. faecium*,\[92\] *E. avium E. gallinarum*, *E. casseliflavus* or *E. raffinosus*.\[25\] But Dupre I et al.\[93\] reported the prevalence of *esp* gene in *E. faecium* (72%) higher than *E. faecalis* (60%).\[83\] The large number of clinical *E. faecium* isolates were enriched with *esp* suggest the role in pathogenicity. He reported the correlation...
between presence of *esp* gene in the clinical enterococcal isolates and they have greater
capacity to adhere to Vero\textsuperscript{93} and Caco-2 epithelial cells in vitro and were less genetically diverse than \textit{esp} negative blood isolates.\textsuperscript{92} There is association between presence of \textit{esp} gene and capacity to grow in presence of ampicillin.\textsuperscript{93}

The gene \textit{esp}, can be exchanged between enterococcal strains. Transfer of \textit{esp} is triggered by selection for conjugative transfer of antibiotic resistance determinants. Mechanisms by which \textit{esp} is transferred vary between species to species, involving integration into a conjugative plasmid in \textit{E. faecium} and by a chromosome-to-chromosome transmission in \textit{E. faecalis}.\textsuperscript{94}

At local sites of microbial infections, platelets, neutrophils or macrophages release large amount of different bactericidal peptides. Ivanov IB reported, all nine strains isolated from patients with chronic prostatitis were highly resistant to human platelet microbicidal protein (hPMP). Nevertheless, very few (1 out 7) isolates from healthy individuals were resistant to hPMP. While similar findings reported by, Yeaman \textit{et al.} showed bacteremic isolates from patients with or without infective endocarditis.\textsuperscript{95} These findings suggest that the phenotypic traits hPMP resistance may be important for bacterial pathogens to induce and perpetuate chronic infection of different localization by surviving or avoiding microbial proteins mediated clearance. The conjugation frequencies increase in the presence of \textit{esp} in \textit{E. faecium}. Majority of \textit{esp}- positive isolates are more resistant than \textit{esp}- negative isolates making these pathogens excellent survivors in hospital environment.\textsuperscript{83} These provide direct evidence of ESP protein in virulence.

3. **Coccolysin**

Around 50-60\% strains of \textit{E. faecalis} produce coccolysin, an extracellular metalloendopeptidase, which may play a role in virulence by inactivating endothelin, a vasoactive peptide.\textsuperscript{19}

4. **MSCRAMM Ace**

Ace is a collagen binding MSCRAMM (Microbial surface component recognizing adhesive matrix molecule) on enterococci and is structurally and functionally related to staphylococcal adhesin. It is present in both commensals and pathogenic isolates of \textit{E. faecalis} and is apparently expressed during infection in human.
Human derived antibodies to Ace can block adherence to extracellular matrix proteins
Employing anti-Ace antibodies, Ace was detected in 90% enterococcal endocarditis patient’s serum samples, suggesting that Ace is expressed in vivo also. An Ace homolog, designated Acm, is identified in *E. faecium*.[21]

Detailed understanding of the pathogenesis of enterococcal virulence factors and their correlation with particular infection is very much useful for future improvement in the prevention and therapy of enterococcal infections.

### 5. BIOFILM FORMATION

The ability to form biofilm on abiotic surfaces is an important virulence property of enterococci.[91] A biofilm is an assemblage of microbial cells associated with a surface and enclosed in a matrix of primarily polysaccharide intracellular adhesin (PIA), which mediates cell-to-cell adhesin.[91,96] Biofilm infections represent a major challenge in the current medical practice. Enterococci have been associated with biofilms in endocarditis, urinary tract infections, root canal infections and ocular infections and in a variety of device related infections in which biofilms are found on artificial hip prostheses, intrauterine devices, prosthetic heart valves, catheters and stents.[91] Although these infections often occur on medical devices, removal of the device is some time unsafe or impractical. Antibiotics can occasionally eradicate the biofilm infections, e.g. intravascular catheter-related blood stream infection.[97] *Enterococcus faecalis* forms biofilm in vitro. It rottenly isolated from biofilms on the surfaces of various indwelling medical devices associated with chronic infection.[98]

Dupre I *et al.*[93] investigated a possible correlation between presence of *esp* gene and the capacity of isolates to form biofilms, the capacity of 47 enterococcal isolates to form biofilms on polystyrene micro titre plates was evaluated. The presence of *esp* gene is good marker for identification of strains that are highly adherent to abiotic surface. There are many tests available to detect the biofilm production by different organisms such as tissue culture plate, tube method and Congo red agar.[81,93]

Antibiotics can occasionally eradicate biofilm infections, e.g. intravascular catheter-related bloodstream infection (CRBSI). It has been shown that mono-therapy with either vancomycin or ampicillin frequently fails to eradicate enterococcal biofilms from patients with CRBSI, when the colonized catheter is left in situ. This failure may
occur even though the causative organisms are susceptible to same antibiotics in vitro.
This suggests that selection of antimicrobial agents for the eradication of microbial biofilms flawed when based on traditional susceptibility testing methods, such as the broth MIC or breakpoint determination and may result in treatment failure.\cite{97}

In vitro susceptibility to ampicillin or vancomycin by routine disc diffusion testing of enterococcal isolates causing intravascular CRBSI was not predictive of treatment success when the catheter was left in situ. MBIC\textsubscript{90} of ampicillin, linezolid and vancomycin were 1000 times greater than MIC\textsubscript{90}, at least twice the MBC\textsubscript{90} and well above serum levels that are achievable or sustainable in patients. Ampicillin and vancomycin are often combined with gentamicin for treatment of serious enterococcal infections based on presumed synergy. The addition of gentamicin to linezolid or vancomycin produced a significant reduction in MBC and MBIC. No antagonism was seen with these antibiotic combinations. Although gentamicin enhanced the effects of vancomycin and linezolid against some \textit{E. faecalis} biofilms, it did not have a marked effect on the activity of ampicillin in this respect. High-level susceptibility to gentamicin by disc diffusion did not necessarily predict a reduction in MIC, MBC or MBIC.\cite{97}

6. **ANTIBIOTIC RESISTANCE DETERMINANTS**\cite{25}

The majority of enterococci exist as commensals in the gastrointestinal tract or in ecologies contaminated by human wastes, with a comparatively minuscule fraction that experience natural selection in the process of causing human infection. The concept that emerges is that they acquire virulence traits which operate in a genetic background that has evolved to survive in the highly competitive gastrointestinal tract. The intrinsic roughness of the enterococcus definitely contributes to its persistence at sites of infection as well as to the organism’s resistance to antibiotics.

Nosocomial enterococcal disease is predominantly a two-stage process. Initially, asymptomatic colonization with nosocomial strains followed by tissue invasion. The second, exogenously acquired factor could enhance enterococcal virulence at the level of tissue destruction or toxicity, enhancing its ability to breach containment in the gastrointestinal tract or other commensal sites and cause symptomatic disease.

Several factors confound the relationship between enterococci, virulence and
disease. Enterococci are leading cause of subacute endocarditis, which typically occurs
in older male patients with genitourinary tract infection. Other enterococcal diseases occur in patients with underlying conditions representing a wide spectrum of severity of illness and immune modulation. If immune-suppression increases, the particular virulence traits enhance the severity of the disease. The enterococcal isolates should contain a spectrum of strains, from pure commensals to those harbouring the most synergistic combinations of various virulence traits.

*E. faecalis* blood isolates were examined for production of a cytolytic toxin, which confers a haemolytic phenotype, as well as for antibiotic resistance. In one study, of 190 isolates, 36% were resistant to high levels of aminoglycosides and 45% were haemolytic. Notably, these traits were not distributed randomly and independently. While, 91% of the gentamicin-resistant strains were haemolytic, where as only 19% of the gentamicin-susceptible strains were haemolytic. Furthermore, genetic identity confirmed that 12 of 12 haemolytic, gentamicin-resistant strains were identical (from 10 different patients) versus only 2 of 9 nonhaemolytic, gentamicin-susceptible strains (from 08 different patients). These data show that neither gentamicin resistance nor the cytolytic toxin conferring the haemolytic phenotype had deeply penetrated the species and imply that the two factors may work synergistically to result in disease.

In a large study that examined the relationships between enterococcal disease and enterococcal traits, isolates related by DNA fingerprint were between 25 and 33% of the presumably unique genetic lineages derived from infection, sites and a slightly lower proportion from the gastrointestinal tract of hospitalized patients possessed the trait of cytolytic toxin production. The actual percentages of infections caused by cytolytic toxin-expressing strains are not yet determined.

The *E. faecalis* approximately have one-half of the genetic lineages, isolated from infections or faeces of hospitalized patients possessed genes for aggregation substance and the protease gelatinase. Aggregation substance is an integral component of the pheromone-responsive plasmid exchange system. Therefore, nosocomial strains of *E. faecalis* may be those best equipped to participate in genetic exchange and might be selected by the presence of antibiotic resistance determinants on such plasmids. The basis for the penetration of the gelatinase determinant into *E. faecalis* lineages associated with the nosocomial environment is more obscure. Gelatinase, aggregation
substance and cytolysin were observed among all faecal strains from the community in approximately 25% of the genetic lineages examined.

The bacteremic patients harbouring strains expressing the haemolytic cytolysin in a background of gentamicin resistance were at a fivefold-increased risk of death within 3 weeks of a culture-positive blood specimen. Although aminoglycoside resistant, all isolates were negative for β-lactamase production and none was vancomycin resistant. Moreover, this risk of mortality is found to be independent of therapy.

Enterococci are also a leading cause of sub acute endocarditis, the rabbit endocarditis model using abraded heart valves, via catheter-induced trauma, was also used to assess virulence. Infused organisms are permitted to colonize the damaged valve, and comparison of various treatment regimens and pathologies can be studied. These models have enabled the key findings in the contribution of various virulence factors to the course and severity of enterococcal disease.

ROLE OF ENTEROCOCCI AS NORMAL FLORA OF HUMAN[12]

The enterococci are the member of the GIT as a commensal microflora of human, occurring in numbers as high as $10^8$ cfu/gm of faeces of adult individuals. Enterococcal population in the intestinal tract fluctuate in size according to the age and physiological condition of the human host, more numerous during early life. Diet also seems to affect the numbers of enterococci in faecal samples.

The enterococci are opportunistic pathogens; the incidence of each species found in human infections probably reflects the distribution of different enterococcal species in the human gastrointestinal tract. This site is believed to represent an important reservoir for strains associated with diseases; from this location, they migrate to cause infections and can disseminate to other hosts and environmental surface.

The comparison of data from different publications and the evaluation of real incidence of the different species of enterococci as member of intestinal microflora in other body sites are different due to difference in the methodology used and change in the taxonomy of the genus.
Enterococci are mainly opportunistic pathogens. They are normal inhabitants of the alimentary canal and cause urinary tract infections, bacteremia and endocarditis. They are also commonly recovered from infections of the abdomen, pelvis, biliary tract and wounds, settings in which polymicrobial flora are common. Enterococci less frequently cause infections of other sites, for example, bone, joints and meninges.[27] The increasing severity of illness in hospitalized patients has contributed to the ascendance of enterococci as nosocomial pathogens. Enterococci are the fourth most common isolate from all sites.[28]

_E. faecalis_ is the predominant enterococcal species, accounting for 80 to 90% of all clinical isolates[99] and _E. faecium_ accounts for 5 to 15%. Other _Enterococcus_ species (_E. gallinarum, E. casseliflavus, E. durans, E. avium, and E. raffinosus_) are isolated much less frequently and account for less than 5% of clinical isolates.[27,100]

### Clinical Infections Caused by Enterococci[11]

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>percentage of cases caused by enterococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary tract infections</td>
<td>&lt; 5% in young and healthy individuals&lt;br&gt;15% in nosocomial UTIs</td>
</tr>
<tr>
<td>Intra-abdominal, pelvic and wound infections</td>
<td>15% frequently isolated but other organisms are more important.</td>
</tr>
<tr>
<td>Spontaneous peritonitis (cirrhosis)</td>
<td>5-10%</td>
</tr>
<tr>
<td>Nosocomial bacteremia</td>
<td>10-15%</td>
</tr>
<tr>
<td>Neonatal sepsis</td>
<td>Unusual</td>
</tr>
<tr>
<td>CNS infections</td>
<td>Rare</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>Rare</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Infection with VRE usually develops in patients colonized with the bacteria, with the ratio of infected-to-colonized patients dependent on the specific patient population. It is highest in haematology patients and organ transplant recipients and approaches zero in healthier (immunocompetent) populations. Portals of entry for VRE typically include the urinary tract, intra-abdominal (e.g. gastrointestinal tract, biliary tree) or pelvic sources, wounds (surgical wounds, decubitus ulcers) and intravascular
catheters.^[101]
1. Urinary Tract Infections (UTI):

Urinary tract infections are the most frequent infections caused by enterococci and now it is recognised as the third most common urinary isolate in the urine culture\cite{28,82} which is the most common source of enterococci in the clinical laboratory. They cause complicated UTI. Enterococci have been associated with cystitis, pyelonephritis, prostatitis and perinephric abscesses, most of these are nosocomial in origin.\cite{101} Enterococci cause UTI, particularly among hospitalized patients.\cite{24} The rate of urinary colonization and infection by enterococci rises in nosocomial UTIs, and are associated with urinary catheterization (8.92%),\cite{23} instrumentation or received antibiotics; as well as who have structural abnormalities, have recurrent UTIs\cite{22} and/or renal transplantation.\cite{102} The prevalence of nosocomial enterococcal UTIs are increasing in number of hospitals. In contrast, enterococci are the rare cause of infection such as uncomplicated cystitis in non-hospitalized women.\cite{1,3} A multicenter study in USA showed that enterococci accounted for 13% of urinary tract infection in hospitalized patients in 1998.\cite{1}

2. Skin Wound and Soft Tissue Infection:

Enterococci are rarely isolated in pure culture from skin and soft tissue infection. They are frequently isolated as mixed culture with Gram negative bacilli and anaerobes from surgical wound infections, decubitus ulcers, diabetic foot infections and burns.\cite{22} They rarely cause cellulitis or deep tissue infections. They have been occasionally found in diabetic and non-diabetic patients with chronic osteomyelitis.\cite{3} Skin and soft tissue infections identified as the source of bacteraemia in 15 to 30% cases.\cite{22}

Enterococcal wound colonization and sepsis have been described in burn patients, whose wounds were covered with porcine xenografts presumably contaminated with enterococci.\cite{3} These infected burn wounds have been found to be a significant source of enterococcal bacteraemia. The rate of bacteremia appears to be increasing, and bacteraemias have been associated with a high mortality rate. Maki and Agger reported that 11 of their 25 patients with enterococcal bacteremia secondary to burn wounds had received oral erythromycin and neomycin as prophylactic antibiotics. Ninety percent of isolates from these patients were resistant to erythromycin and neomycin, again emphasizing the selective pressure of antibiotic in increasing the incidence of enterococcal infections.\cite{22}
3. Intra-Abdominal and Pelvic Infections:

The enterococci are found in the faeces of most of the adults and routine vaginal cultures. The second most frequent enterococcal infections are intra-abdominal and intra-pelvic abscesses or post surgery wound infections.\textsuperscript{[3,26,28]} They are commonly isolated from postoperative infections after penetrating abdominal trauma unless there was gastrointestinal perforation. The clinical manifestations of enterococcal intra-abdominal infections are similar to those caused by other organisms. Enterococcal infections were common when intra-abdominal and gynaecological infections were treated with cephalosporin.\textsuperscript{[22]} They have also caused acute salpingitis, peripartum maternal infection (such as endometritis) with bacteraemia and abscess formation following caesarean section. Enterococcal abscesses and bacteremia also occur with intra-abdominal and biliary infections.\textsuperscript{[3,15]}

Enterococci can cause primary peritonitis in adults and children.\textsuperscript{[1]} They have been reported as a cause of spontaneous peritonitis in both cirrhosis, nephrotic syndrome and can cause peritonitis in patients undergoing chronic ambulatory peritoneal dialysis.\textsuperscript{[15]} Thierry De B et al. reported few cases of chronic ambulatory peritoneal dialysis caused by \textit{E. cecorum}.\textsuperscript{[55]} “Pure” enterococcal peritonitis is also seen occasionally as a complication of abdominal surgery or trauma.\textsuperscript{[3]}

In these infections, enterococci are usually part of a mixed aerobic (\textit{E. coli}) and anaerobic (\textit{Bacteroides} species)\textsuperscript{[1,3]} flora commonly found in the GIT. Parvati et al. reported that 43% of enterococcal isolates were in combination with other bacteria.\textsuperscript{[15]} The exact role of enterococci in mixed intra-abdominal and pelvic infections remains murky.\textsuperscript{[3]} In an experimental intra-abdominal abscess model, enterococci injected into peritoneum, as a single organism did not cause any abscess or death. However, when enterococci were injected along with anaerobes, the combination caused abscess formation in 89 to 95% of animals. This suggests that enterococci are pathogenic only in synergistic combination with anaerobic bacteria. Additionally, antibiotic regimens with no activity against enterococci have shown to be effective in treating intra-abdominal infections even when enterococci have been isolated. However, these intra-abdominal sites are frequent source of bacteremia. When the bacteremia arises from an intra-abdominal sites, the mortality rate is high, may be up to 40%. There are several reports of intra-abdominal infections, including peritonitis; in which enterococci was the
sole organism isolated.\textsuperscript{[22]}
4. Blood Steam Infection:

Many studies all over the world showed that enterococci are responsible for sepsis. It varies in different center and different part of the world. Many factors like geographic, socio-economic, use of ventilators and administration of different antibiotics which play an important role in explaining these differences. Septicaemia is a major cause of mortality and morbidity among the neonates around the world. The incidence of Enterococcus faecalis in blood stream infection is increasing.\textsuperscript{[42]}

**Bacteraemia:** Enterococcal bacteraemia is increasing in frequency possibly due to antimicrobial pressure. In most of the studies, the enterococci are (second in US hospitals)\textsuperscript{[82]} third \textsuperscript{[16,28,104,105]} or fourth\textsuperscript{[1]} most frequent organism to cause blood stream infections especially bacteraemia. In 1986-1990 according to NNIS system surveillance, enterococci are reported to cause 8.3\% of cases of nosocomial bacteraemia.\textsuperscript{[22]} Incidence of *E. faecalis* appears to be increasing day by day.\textsuperscript{[42]} Enterococcal bacteraemia is much more common than enterococcal endocarditis.

The source of enterococcal bacteraemia without endocarditis is often the urinary tract infection (19-43\%), intra abdominal sepsis, hepato-biliary tract infection, vascular and catheters.\textsuperscript{[1]} Enterococcal strains have been reported as a major cause of secondary bacteraemia in patients with gynaecological and postoperative wound infection. The soft tissue infections (15-30\%) are another major source of bacteraemia.\textsuperscript{[22]}

The majority of cases of bacteraemia due to *E. gallinarum* and *E. casseliflavus* involved patients with underlying conditions, such as renal failure, solid organ cancer or haematological malignancy, receipt of solid organ or bone marrow transplant, antithrombin III deficiency, astroctoma, chronic osteomyelitis, diabetes mellitus, choledocholithiasis and Caroli’s disease.\textsuperscript{[35,106]}

Enterococci are associated with other bacteria in 25 to 45\% of bacteraemia cases. The clinical manifestations of enterococcal bacteraemia are influenced by whether enterococci are isolated alone or as a part of polymicrobial bacteraemia. When bacteraemia is solely caused by enterococci, is typically an indolent disease, frequently characterised by only fever and minimal local infection. Bacteraemia is rarely associated with disseminated intravascular coagulation (DIC) or shock. Polymicrobial bacteraemia is more associated with the development of shock (50\%),
thrombocytopenia or DIC (33%)."^{22}
Endocarditis: Enterococci have been recognized as an important cause of endocarditis for almost a century. The first case of the endocarditis due to *Micrococcus zymogens* (β-haemolytic enterococci) has reported in 1899. Now enterococci are the second or third most common cause of endocarditis. They cause an estimated 5 to 20% of bacterial endocarditis. *E. faecalis* followed by *E. faecium* and other species cause endocarditis. Motile enterococci like *E. gallinarum* and *E. casseliflavus* are not frequently recovered from clinical specimens but may cause serious invasive infection in humans, especially immuno-compromised and chronically ill patients. The enterococcal endocarditis occurs more commonly in older individuals, rarely in infants and occasionally in children.

A source of enterococci is usually not found; however, in many cases common risk factors for enterococcal endocarditis included genitourinary instrumentation, urinary tract infections or abortion (38 to 50%). In women, enterococcal endocarditis occurs during the childbearing age. Mandell and colleagues found that 50% of men with enterococcal endocarditis had previous history of enterococcal UTI or genitourinary tract instrumentation and 43% of women had history of childbirth or a genitourinary tract procedure in the preceding three months. Biliary tract also sometimes acts as a source of enterococci for enterococcal endocarditis.

The infection can occur on valves that are apparently normal as well as on those with underlying diseases. Enterococci account for 5 to 20% cases of native valve endocarditis. The patients with underlying valvular heart disease (42%) are at great risk for developing enterococcal endocarditis. Nevertheless, Maki and Agger also found that community acquired enterococcal bacteraemia was associated with a much higher risk of endocarditis. Bacteraemia with enterococci as the single isolate also act as a risk factor for endocarditis. Intra venous drug use also appears to predispose to enterococcal endocarditis. They can also cause endocarditis in drug addicts (5 to 10%). The valves most often involved in drug addicts are mitral or aortic valves.

Enterococcal endocarditis occurs more commonly in the community acquired, but it may be hospital acquired also. It endocarditis is usually subacute or may be acute, with rapidly progressive to valve destruction. Antibiotic resistance in hospital strains of enterococci have decreased therapeutic options. Endocarditis due to high-level gentamicin resistant enterococci has been reported. Possible endocarditis & bacteraemia
due to β-lactamase-producing and vancomycin resistant enterococci have also been reported. Now days prevalence of these resistant strains is increasing in endocarditis cases.[22]

5. Neonatal infections:

According to recent reports, nosocomial infections in NICUs have been attributed to variety of organisms, including *Klebsiella pneumoniae, Pseudomonas cepacia, Flavobacterium meningosepticum* and *Streptococcus agalactiae*. Although group B streptococci and *Escherichia coli* are the most common causes of neonatal infections, it is well documented that enterococci can also cause infection in the normal infants.[1] Neonatal bacteraemia and sepsis due to enterococci have been reported to be increasing in incidence, accounting for approximately 13% of bacteriologically confirmed cases of neonatal sepsis and meningitis. Enterococcal sepsis is more common in neonate with a low birth weight (mean 913 gm) with less gestational age[108] (mean 27 weeks).[22]

The risk factors for enterococcal sepsis included prolonged non-umbilical central venous catheterization and bowel resections.[22] The infants with severe underlying disease, intravascular device, umbilical arterial or venous catheters, nasogastric endotracheal intubation and prematurity appear to be more susceptible.[1,3,108] Nosocomial transmission has also been documented among a large number of infants. There are several epidemic nosocomial outbreaks of bacteraemia or meningitis or both reported due to *Enterococcus faecium* or *E. faecalis* in neonates.[3] The epidemic organism was spread via the hands of hospital persons.[22] The enterococci are nosocomial pathogen in late onset neonatal sepsis.

The common symptoms of enterococcal sepsis in neonates are respiratory distress, poor feeding and lethargy. Infections are also associated with jaundice, irritability, fever and decreased muscle tone accompanied by bacteraemia and meningitis.[3,22] Leukocytosis may or may not be present. Meningitis may be present; six of 11 had cerebro-spinal fluid cultures positive for enterococci in one study. The development of meningitis has been associated with central nervous system defects or prior neurological procedures. Ventriculo-peritoneal shunts particularly predispose to enterococcal meningitis in neonates.[22] In general, neonates with enterococcal sepsis have responded well with appropriate antimicrobial therapy.[3]
6. Central Nervous System (CNS) Infections:

Enterococcal infections of the CNS are uncommon but have been described in all age groups. In addition to neonatal meningitis, enterococci can also cause central nervous system infections in older children and adults. The predisposing factors are the patients with anatomical defect in central nervous system, underlying long-term primary diseases, invasive procedures of the central nervous system and prior therapy with antimicrobial agents. The enterococcal meningitis rarely develops in patients who have underlying complications like meningeal leukaemia while receiving intrathecal chemotherapy; after basilar skull fracture and after intra-cranial surgery. Enterococci have also been reported as a cause of central nervous system shunt infections, particularly those that terminate in the peritoneum (2 of 19 ventriculo-peritoneal infections) or ureters (2 of 14 ventriculoureteral infections and 1 of 9 lumboureteral infections). Meningitis is a rare complication of high grade bacteraemia in patients with enterococcal endocarditis. Enterococcal meningitis appears to be associated with low cerebrospinal fluid leukocyte counts (usually less than 200/mm³) in most but not all patients.

7. Nosocomial Infections and Super-infections:

Enterococci began to be recognized as common cause of hospital acquired infections in the middle to late 1970s. Currently they are emerging nosocomial pathogens. The Center for Disease Control and Prevention's National Nosocomial Surveillance survey listed the enterococci as the third most common cause of nosocomial infections and second most common cause of nosocomial UTI and wound infection (1990s). They reported enterococci were responsible for about 10% of nosocomial infections, 9% of bacteraemia and approximately 16% of UTIs.

In most instances of nosocomial enterococcal disease, it is not known, whether the organism comes from the own flora of the admitted patient or the organism is acquired following hospitalization. Although, some early studies did not suggest transmission within the hospital, but more recent works, including studies of outbreaks among patients (neonates), has demonstrated person-to-person spread. The use of
antimicrobial agents lacking enterococcal activity is implicated as important factor in
the development of enterococcal superinfection. The spectrum of diseases ranges from asymptomatic bacteriuria to pyelonephritis.\textsuperscript{[1]}

There are progresses in medical technology and treatment, such as the use of various intravascular access devices, implanted prosthetic devices, cytotoxic chemotherapy and immunosuppressant. These have magnified the impact on organisms, which are relatively of low virulence, such as enterococci. Of critical importance is the intensive use of relatively broad-spectrum antibiotics in the hospital, which provides selective pressure favouring the growth of intrinsically drug-resistant commensal organisms such as enterococci.\textsuperscript{[28]} Several factors appear to be involved in the emergence of enterococci as an important nosocomial pathogens, including the multiple resistance characteristic of these organisms, use of cephalosporin and other antimicrobial agents to which organism is resistant, use of mechanically compromising devices such as intra vascular lines and urinary catheters as well as increasing numbers of seriously ill and debilitated patents in hospitals.\textsuperscript{[1]}

Pneumonia rarely caused by enterococci and other infections are reported in patients treated with moxalactam. Enterococcal pneumonia is also seen following the use of polymyxin aerosol for prevention of \textit{Pseudomonas aeruginosa} pneumonia.\textsuperscript{[25]}

Nosocomial enterococcal infection has also been reported in transplant patients. A recent paper from England reported an unusually high rate of enterococcal bacteraemia in liver transplant patients.\textsuperscript{[25]}

Further, enterococci with high-level aminoglycoside resistance (HLAR), beta lactamase production and glycopeptides resistance including vancomycin resistant enterococci (VRE)\textsuperscript{[25]} have emerged posing a therapeutic challenge to physicians due to the ease of acquiring and transferring antimicrobial drug resistance.

8. Other Miscellaneous

\textbf{Infections:}

\textbf{Endophthalmitis:}

The endogenous \textit{endophthalmitis} is an unusual ocular infection caused by enterococci.\textsuperscript{[22,38]} There are some reports on enterococcal endophthalmitis in diabetic
patient who had enterococcal bacteraemia from diabetic foot infection several months after undergoing vitrectomy for diabetic retinopathy.[3]
Respiratory Infection:

Enterococci are rarely implicated as the cause of lower respiratory tract infection. Although, isolation of enterococci in approximately 2% of respiratory cultures, these organisms are generally assumed to be non-pathogenic colonisers.[22] Although well documented cases of enterococcal pneumonia and even lung abscess exist, they usually occur in patients with underlying severe and debilitating diseases. There have been reports of pneumonia in patients receiving broad-spectrum cephalosporins coupled with enteric feeding.[3,22] The association of enterococci with antibiotic therapy suggests that enterococcal pneumonia may increase in future years.[22]

Pericarditis:

It is usually secondary to extension of underlying conditions such as infection from pleural cavity or pleural effusion. Combined antibiotic therapy and daily drainage from pericardium and pericardiectomy may be required for recovery in these patients.[38] Gallbladder, biliary tract infection[109] and otitis media with effusion are also rarely caused by enterococci.[22]

Association between Infections and Mortality:

Mortality due to enterococcal UTI, in the absence of bacteraemia, is low.[22] Mortality of enterococcal bacteraemia is generally high, in patients with underlying complicating factors. Nevertheless, actual mortality associated with bacteraemia is difficult to assess; overall mortality of enterococcal bacteraemia is around 30-68%.[3,22]

Recipients of autologous peripheral blood stem cell transplants have shown mortality rates as low as 10%. Patients with endocarditis caused by VRE have higher mortality rates, especially those with solid tumours (>50%) and critically ill and liver transplant patients (70%) have higher mortality rates.[101]

The mortality risk associated with VRE bacteraemia vary according to the study design and analysis, patient population at risk,[101] case definition, control selection and enterococcal species studied. The crude mortality rate among bacteremic patients with E. faecium does not differ between those with vancomycin-susceptible and vancomycin-resistant isolates. Shales and colleagues found a mortality rate of 34% in 74 patients with enterococcal bacteraemia. Most of these infections occur in setting following instrumentation or surgery.[116] Virulence traits are less characterized in E. faecium compared to E. faecalis, neither group of investigators were able to assess the blood...
isolates for potential virulence traits that may have contributed to mortality.\textsuperscript{[25]}
The strains with normal haemolysin phenotype are significantly more virulent (higher mortality rate) than non-haemolysin strains. The presence of haemolysin-bacteriocin is not sufficient to increase the mortality unless accompanied by the ability of the strain to produce aggregation substance. The mortality is increased in strains containing “wield-type” indicating the presence of both haemolysin and aggregation substance.\textsuperscript{[18]}

During the management of infectious disease, we should keep in mind that more antimicrobial drug resistances equate with greater virulence. Attributable mortality is linked to antimicrobial susceptibility profile rather than the availability and prompt initiation of suitable antimicrobial agents. Mortality is associated with multidrug-resistant organisms. There is a strong association between high level gentamicin resistance and expression of a known enterococcal cytolytic. Infection with cytolytic and high level gentamicin resistant strains was responsible for fivefold-increased risk of death in spite of the even vancomycin susceptibility of these isolates.\textsuperscript{[18]} There is an association between cytolysin production and severity of illness.\textsuperscript{[25]}

The enterococci are not actually more virulent organisms, but they cause infections in severely debilitated, immune-compromised patients which increase the possibilities to cause the death of patients. In many cases, morbidity and mortality is difficult to assess because enterococci are the part of poly microbial infections.\textsuperscript{[3]}

Clearly, further clinical and translational research will need to dissect such questions for enterococci, including species other than \textit{E. faecalis} and about host immune responses to enterococci. Collaborative works on the clinical assessment of patients and the molecular characterization of recovered isolates will be helpful for our understanding of enterococcal virulence.\textsuperscript{[25]} Despite the low morbidity and mortality associated with enterococcal infections (UTIs), these have been associated with increased length of stay & cost of hospitalization.\textsuperscript{[22]}
Epidemiological studies have been hampered by the lack of a simple, convenient, reliable and accessible method for distinguishing individual strains. Biotypes and antibiograms often show little variation within enterococcal species. Bacteriophage typing, enterococccin typing and molecular typing have used with some success but require access to special reagents and performance of a large number of tests.\textsuperscript{[19]}

Isolation, identification and comparison of different enterococcal species may be helpful in epidemiological studies. Many persons infected with a single strain would suggest that an outbreak has occurred, while the presence of different strains would point away from an outbreak.\textsuperscript{[110]} \textit{E. faecalis} is the most prevalent (80 to 90\%) species isolated from human clinical specimens in India\textsuperscript{[35]} as well as other parts of the world.\textsuperscript{[111]} The prevalence of non \textit{E. faecalis} and non \textit{E. faecium} enterococci is around 2 to 10\% in India but Vitthal PP et al.\textsuperscript{[112]} reported 19\% prevalence in south India. This prevalence of non \textit{E. faecalis} and non \textit{E. faecium} enterococci was underestimated because of frequent misidentification.

In the 1990s, enterococci became the third most common pathogen bloodstream infections causing among hospitalized patients, with \textit{Enterococcus faecium} and \textit{Enterococcus faecalis} being the predominant species. Recent data indicate that up to 50\% isolates of \textit{E. faecium} are resistant to vancomycin. Vancomycin-resistant enterococci (VRE) present a considerable therapeutic challenge because vancomycin resistant \textit{E. faecium} is also resistant to several key antibiotics.\textsuperscript{[113]}

**Shifting of Spectrum Enterococcal Infection:**

Over the last six decades, the patterns of microorganisms causing nosocomial infections have shown dramatic change. These changes reflect a shift from the more easily treated pathogen towards more resistant ones leaving fewer options for therapy. Since 1918s, Gram positive organisms had emerged as important nosocomial pathogens. Enterococci had emerged during 1990s as nosocomial pathogen and are now regarded to be the second leading cause of such infections.\textsuperscript{[5]}
Epidemiologic studies have shown the association between enterococcal species and disease caused by them. The microbiological shift might be due to emergence of VRE, in particular, the predominance of the species *E. faecium* among this subset of
enterococcal isolates. Firstly, these organisms have been shown to play an increasing role in nosocomial infection and secondly, they seem to possess a remarkable and increasing resistance of enterococcal isolates from nosocomial infections than those from community acquired.\textsuperscript{[5]} Data from the National Nosocomial Infection Surveillance (NNIS) survey reveal a rising percentage of VRE since 1989, with rates now approaching 20\% of all enterococcal isolates (including all species). There is an equal proportion of VRE isolates occurring in and out of intensive care units and \textit{E. faecium} as the dominant species identified among VRE (although many enterococci did not identify). There was a 47\% increase in VRE from 1994 to 1999 (NNIS).\textsuperscript{[25]} The vancomycin resistant enterococci were not found in initial studies of animals or in the faecal flora of healthy persons from the community but according to recent reports VRE have been recovered from animal feeds.\textsuperscript{[114]} The increasing VRE, MDRE and nosocomial infections caused by them have been reported from all parts of the world.\textsuperscript{[5]}

\textit{E. faecalis} resistance is low against vancomycin and ampicillin while resistance among \textit{E. faecium} isolates is high (60 \& 80\% respectively) and rising. The prevalence of motile enterococci (like \textit{E. gallinarum} and \textit{E. casseliflavus}) is less than two percent. They are intrinsically resistant to vancomycin and inappropriate therapy with vancomycin may contribute to higher morbidity and mortality.\textsuperscript{[115]}

**Geographic Distribution**

VRE have been recovered from patients in the United Kingdom and France, as well as from many other countries, including Australia, Belgium, Canada, Denmark, Germany, Italy, Malaysia, Netherlands, Spain, Sweden and United States.\textsuperscript{[27,115]} However, in India, the incidence of enterococcal infection is not thoroughly investigated. Isolation of \textit{E. faecalis} was increasing in nosocomial infections, reported to the Centres for Disease Control and Prevention’s National Nosocomial Infections Surveillance System.\textsuperscript{[31]} These increasing prevalence was mainly due to rise (from 0.4 to 13.6\%) of VRE infections in intensive care unit (ICU) and non-ICU patients.\textsuperscript{[27,115]}

The geographic differences might be due to the use of glycopeptide (avoparcin) as growth promoter in animal feeds (licensed since 1975) in some European countries. It has been fed to many animals like broiler chickens, cattle and swine. The Avoparcin causes cross-resistance to vancomycin and teicoplanin among bacteria. Here animal to
human food chain appears as an important factor in vancomycin resistance, based on periodic examination of sewage and on comparisons of manure of animals fed with or without antibiotic (avoparcin) growth promoters (these organisms readily colonize the intestinal tract of animals for which avoparcin was used as a feed supplement). However, the greater incidence of VRE is also reported in the US hospitals. Even though, in the US avoparcin is not a licensed (and used) feed additive for animals and culture surveys of a limited number of chickens in several cities have failed to detect VRE. In fact, there are few evidence to suggest that transmission of VRE occurs in healthy adults in the US community. VRE are recurrently establish in the gastrointestinal tract of human and have become progressively more responsible for hospital acquired infections, particularly in the USA. In Europe, however, VRE have tended to be associated with community carriage and occasional causes hospital acquired outbreaks.

The use of vancomycin in the US hospitals has increased dramatically in the past 10 to 15 years. The imprudent use of antimicrobial agents and the increasing colonization pressure are the major contributors to selection of vancomycin resistance. In the US, hospitalized patients with gastrointestinal carriage of VRE appear to be the major reservoir of the organism and cause an endogenous infection. This might describe the significance of VRE as a causative agent of catheter-related sepsis. It may enhance the risk of cross-infection or blood culture contamination.

There is also evidence for direct exogenous acquisition of infection. Oropharyngeal colonization may provide a source for cross-colonization, particularly if hospital staffs do not wash their hands after tracheotomy or endotracheal tube care. Additionally, skin colonization associated with diarrhoea or faecal incontinence has been reported.

Environmental surfaces and medical equipment items in the patient’s room frequently become contaminated with VRE. This may serve as a reservoir for it especially when patients from same room have diarrhoea. The various items that may be contaminated include patient gowns and linen, beds, bedside rails, over-bed tables, doorknobs, washbasins, floors, glucose meters, blood pressure cuffs, electronic thermometers, ECG monitors, ECG wires, etc. VRE may remain viable on these
contaminated surfaces for few days or weeks and may act as a source from which health care workers (HCWs) may contaminate their hands or clothing.[115]

Once colonized, the patients remain so for weeks or months (even up to 1 yr). Thus, as colonized patients leave the hospital environment, the possibility that transmission might occur in the community cannot be discounted.[71] Another aspect to be keep in mind is that hospitals should develop means of prompt identification of such patients at the time of readmission so that they can place them in isolated room right away.[115]

The spread of glycopeptide resistance among enterococci is an epidemic of genes that are mobile to varying degrees and an epidemic of clones carrying those resistance determinants. A recent study suggested that an epidemic clone of VRE carrying a putative virulence determinant was present in outbreaks in 3 continents. As individual strains of VRE are identified by use of pulsed-field gel electrophoresis (PFGE), the gene clusters themselves can be analyzed and tracked by DNA-based techniques.[28]

Hospital outbreaks of infection or colonization reported with both VanA and VanB isolates. Such outbreaks may involve clonal dissemination of strains indistinguishable by PFGE, not only within hospitals but also among several local hospitals. Some nosocomial outbreaks are monoclonal or oligoclonal[117] whereas other analyzes show multiple clones. Single clone has been noted in multiple medical centres in a same city, and in some cases, in different states. The molecular epidemiology of VRE within an institution may change over time, with certain clones establishing themselves as vancomycin resistance becomes endemic.[28]

Multiple clones and sporadic isolates of unrelated strains may coexist with a predominant clone suspected of institutional spread. In hospitals in which VRE outbreaks have been detected at an early stage, cases often caused by a single strain.[19] When VRE have been present in a hospital or community for months or years, molecular typing of isolates frequently reveals that vancomycin resistance has spread by plasmids or transposons to many different clones.[27]

Patients may be colonized simultaneously with one or more than one strain of VRE. Stool isolates of VRE have included a number of diverse species such as E.
faecalis, E. faecium, E. casseliflavus, E. gallinarum, E. avium, E. mundtii etc. Fortunately, rates of stool colonization with VRE among hospitalized patients far exceed infection rates with these organisms. Gastrointestinal tract colonization with VRE may persist for weeks or months, and single negative cultures may be intermixed with positive cultures over time. During outbreaks, environmental cultures in hospital rooms have yielded VRE.[27]

Vancomycin Resistant Enterococci:

Various epidemiological parameters are responsible for the spread of VRE. In Europe, animal husbandry and now community ecologies act as primary reservoirs and sources of VRE strains. While in the United States, VRE reservoirs include hospital staff (nurses), patients and including those who have survived hospital stay; vectors such as stethoscopes, electronic thermometers, sphygmomanometers and health care worker’s hands[25] transmit organisms.

The use of avoparcin (glycopeptide) as a growth promoter in animal feeds seems to be the major contributor to vancomycin resistance. The organisms readily colonize intestinal tracts of animals for which avoparcin was used as a feed supplement. Subsequent enteric colonization of humans has been documented. Now vanA-mediated VRE is ubiquitous in European communities. In contrast, the United States has not permitted the use of avoparcin in animal feed. VRE was not found in the initial studies of animals or in the faecal flora of healthy persons from the community. The use of vancomycin in human’s infections has increased more than 100 fold in the past 30 years. In this country, emergence of VRE and spread appears to result from selection in clinical settings. The indiscriminate use of antimicrobial agents and rising colonization pressure are the largest contributors to selection of vancomycin resistance.[25,117]

Whether VRE in animals are an important contributor to VRE in human infections remains controversial. However, the identity of some of the vanA gene clusters in some animal and human strains, the transferability of these genes from strain to strain and documentation of occasional specific strains shared by human and animals suggest that at least some of the VRE in human come from animals.[117]

According to the earlier reports, VanA phenotype strains of E. faecium were
associated with outbreaks in special units, patients with immuno-compromised and on
prolonged antimicrobial therapy, with extended lengths of hospital stay and higher severity of illness. The lengths of stay, severity of illness and colonization pressure were independent predictors for VRE colonization and infection. Now the enterococcal problem has become endemic, with community-based issues of animal food supplies and nosocomial issues of colonization pressure and severity of illness.\(^{[25]}\)

Enteric VRE colonization usually precedes infection. The lower intestinal tract is the most frequently colonized site and prominent VRE reservoirs are those with frequent hospital readmit. Skin contamination in these patients occurs readily. Numerous studies have demonstrated contamination of health care worker’s gloved and un-gloved hands. Health care workers (29%) may have VRE on their hands after glove removal. Complications of skin colonization such as enterococcal pseudo-bacteraemia are due to injudicious use of antibiotics for therapy. Therefore, the horizontal transmission of VRE in nosocomial environments can occur readily if Centres for Disease Control and Prevention guidelines to prevent the spread of vancomycin resistance genes are not strictly followed.\(^{[25]}\)

VRE in Long-Term-Care Facilities:

The role of long-term-care facilities (LTCFs) in the epidemiology of VRE is not well defined. In Chicago, where VRE was endemic for several years, found that 47% of patients admitted to a hospital from LTCFs were colonized with VRE. The prevalence of VRE among LTCF residents varies significantly depends upon geographic area. Several preliminary reports showed that 10 to 16% of patients admitted to the hospital from nursing homes may be colonize with VRE.\(^{[27]}\)

Bonilla et al. reported that VRE colonization of residents in a Veteran’s Affairs (VA) LTCF in Michigan ranged from 9% (in December 1994) to 19% (in January 1996). During the surveys, it has found that 26 to 41% of health care workers carried VRE on their hands. While in Pittsburgh, vancomycin-resistant \textit{E. faecium} was identified in 24 of the 36 patients at the time of transfer from an acute-care facility. VRE in these patients persisted for a median of 67 days after identification. Treatment of VRE colonization with antimicrobials prolonged colonization. Serial surveillance of the 34-beded ward showed that the rates of colonization were stable, with only three documented instances of VRE acquisition. The authors of this report also noted that
during 2.5 years of surveillance for infection, a single case of bacteraemia occurred in a patient in whom colonization with VRE could not be demonstrated by rectal swab culture and no infections occurred in patients colonized with VRE. These studies indicate that colonized residents of LTCFs may serve as a reservoir for VRE for acute-care hospitals, just as patients from acute-care hospitals may reintroduce VRE to LTCF continuously.[27]

**VRE in the Community:**

There are few evidences to suggest that transmission of VRE among healthy community. Two cases of apparent community acquired VRE urinary tract infections in New York City have been reported. In another case, the husband care giver of an elderly woman colonized with VRE developed urinary retention and urinary tract infection with a VRE strain that has found to be indistinguishable from the woman’s isolate by PFGE. Thus, as colonized patients leave the hospital environment, the possibility that transmission might occur in the community cannot be discounted.[27]

The situation in Europe is quite different from that in the United States. In Europe, VRE have been isolated from sewage and various animal sources. It has been suggested that the use of glycopeptides (e.g. avoparcin) in animal feeds in some regions of Europe may have contributed to such differences. In one study, VanA-resistant *E. faecium* was isolated from frozen poultry and pork and from the faeces of 12 of 100 non-hospitalized inhabitants in a rural area. VanA VRE has also been found in the faeces or intestines of other farm animals or pets, including horses, dogs, chickens and pigs. These observations suggest a potential for VRE or the resistance genes of VRE to reach humans through the food chain or through contact with domesticated animals. It leads to a ban on glycopeptides use in animals feeds in Europe.[27,117]

Colonization of healthy individuals with VRE does not necessarily indicate a risk of infection with these organisms. In a point-prevalence culture survey in Belgium hospital in 1993, showed that 3.5% of patient stool isolates were positive for VRE; however, to that point no infections due to VRE had encountered in that institution. Van Der Auwera *et al.* reported that vancomycin-resistant *E. faecium* were isolated from stool cultures of 28% healthy volunteers who were neither health care worker nor taken
antibiotics in the preceding year. The same group also detected VRE in the stools of up to 64% of volunteers who had received oral glycopeptides.\textsuperscript{[38]}

## Risk Factors

Early studies dealing with the emergence of enterococci in the United States revealed that most patients with VRE were from ICUs. However, colonization and infection with VRE have now seen with increasing frequency among patients with chronic renal failure, renal insufficiency\textsuperscript{[27]} or urinary catheter.\textsuperscript{[31]} While gastrointestinal tract colonization and the use of enteric tube feedings or sucralfate may precede infection in many patients. A stool surveillance culture positivity detected infection in only half of the cases. This may in part reflect the limitations of surveillance cultures in detecting low densities of microorganisms. Occasionally, VRE have been detected in cultures of nose, throat or mouth specimens in the absence of detectable rectal or perineal colonization.\textsuperscript{[27,28]}

Several studies have used case-control methods and multivariate analysis to examine the risk factors for VRE infection among hospitalized individuals. Among the risk factors that have emerged are longer duration of hospitalization,\textsuperscript{[33,101]} length of stay prior to surgical ICU admission, more than one prior ICU stay, longer lengths of stay in ICU and the need for intra-hospital transfer to another ward. Other risk factors that have been associated with colonization or infection include exposure to contaminated medical equipment such as electronic thermometers, proximity to a previously known VRE patient and exposure to a nurse who assigned on the same shift to another known patient.\textsuperscript{[27]}

Karlene CR \textit{et al.} reported in his study various underlying conditions; VRE present in 95% of patients; these condition include malignancy, receipt of solid organ & bone marrow and patient having diabetes mellitus, choledocholithiasis with cholecystolic fistula, obstruction in bile duct or liver abscess and Caloli’s disease with congenital hepatic fibrosis and polycystic kidney disease.\textsuperscript{[35]}

Enterococci have been isolated from various clinical specimens and from patients who are either chronically ill or immunosuppressed.\textsuperscript{[35]} Risk factors specifically associated with VRE infections such as bacteraemia includes malignancy, non-Hodkins lymphoma,\textsuperscript{[36]} lymphatic lymphoma, pancreatic adenocarcinoma, gut
translocation\textsuperscript{[101]} cholangitis, hematologic malignancy,\textsuperscript{[45,101,115]} neutropenia.\textsuperscript{[101,117]} The need for surgical re-exploration followed by history of solid-organ transplantation such as renal, liver, bone marrow transplantation, organ transplant recipients,\textsuperscript{[37,101]} are also the known risk factors for enterococcal infection.\textsuperscript{[27,28]} The underlying disease like diabetes and alcoholism are also reported as risk factors in India.\textsuperscript{[27]} Numerous reports have appeared on the serious infections and mortality associated with these strains, especially among immunosuppressed patients or those underlying illnesses and patients who experience prolonged hospitalization.\textsuperscript{[3,115]} Fortunately infection is not always fatal.\textsuperscript{[37]}

**Role of Previous Antimicrobial Therapy in VRE Acquisitions:**

Risk factors that have been associated with colonization or infection include previous antimicrobial therapy, especially preceding therapy with agents active against anaerobes, number of days on antibiotic therapy. Parenteral vancomycin use and receipt of third generation cephalosporin\textsuperscript{[31,36,37,115]} and aminoglycoside\textsuperscript{[3,22]} have been cited as risk factors for colonization or infection with VRE by some others.

Epidemiological studies have not yielded uniform results in this regard and some authors have concluded that, after statistically controlling the length of hospital stay, exposure to vancomycin is not a risk factor for nosocomial VRE.\textsuperscript{[28]} The rapid spread of vancomycin resistance among enterococci, particularly in *E. faecium*, is well known phenomenon. Because of this, VRE infection cannot be effectively treated with currently available antimicrobial agents. The treatment with imipenem has shown to select ampicillin resistant enterococci.\textsuperscript{[22]} Other antibiotics, including aztreonam and ciprofloxacin have been associated with nosocomial enterococcal infections.\textsuperscript{[3]}

**Vancomycin:**

Hospital based studies have found association between colonization with VRE and oral or parenteral administration of vancomycin\textsuperscript{[117]} and this has led to the recommendations to discourage the use of this agent for the primary treatment of antibiotic associated diarrhoea. The exact consequence of oral or intravenous vancomycin exposure on the acquisition of VRE remains controversial. While few clinical studies explain the role of oral vancomycin in VRE acquisition, oral vancomycin use most likely exercised some initial selection pressure, contributing to
the emergence of this type of resistance. Indeed, one of the first documented cases of VRE has described in a patient who received oral vancomycin.\textsuperscript{[115,118]}
In contrast, many reports describe an association between prior intravenous vancomycin use and VRE colonization or infection; whereas others found no such effect. A recent meta-analysis from Carmeli et al. analyzed 20 epidemiologic studies and showed in a crude analysis that vancomycin exposure conferred a 4.5 fold increased risk of VRE acquisition. Those studies that used patients with vancomycin susceptible enterococci as controls found a stronger association than those that used controls from whom no VRE was isolated. Studies, which were designed according to good epidemiological standards and adjusted for length of stay (LOS) found only a small and no significant association between vancomycin treatment and VRE acquisition. These investigators also detected publication bias, favouring reports that found a large measure of association. They concluded that, the strong association between vancomycin treatment and hospital acquired VRE reported in the current literature might be due to flaws in the selection of the control group, confounded by duration of hospitalization and publication bias.\textsuperscript{[118]}

Vancomycin presumably predisposes patients to colonization and infection with VRE by inhibiting the growth of the normal Gram positive bowel flora and by providing a selective advantage for VRE that may be present in small numbers in the individual’s bowel. For example, the administration of oral vancomycin or teicoplanin to individuals whose baseline stool specimens contained few or no detectable VRE led to recovery of VRE in large numbers (0 to 64%),\textsuperscript{[117]} sometimes as much as $10^6$ to $10^8$ cfu/gm of stool. The selective pressure exerted by the increasing use of vancomycin in the United States during the last 25 to 30 years has been extraordinary. For example, the amount of vancomycin used at one university hospital increased 20 fold from 1981 to 1991.\textsuperscript{[27]}

Beezhold et al. reported a high prevalence of skin colonization with VRE (86%) among hospitalized patients with VRE bacteraemia. Skin colonization with VRE was found to be associated with diarrhoea (prior or present) or faecal incontinence. The high prevalence of skin colonization might explain the importance of VRE as a cause of catheter related sepsis. It may also increase the risk of cross-infection or blood culture contamination, which may also explain the frequent spontaneous resolution of bacteraemia due to VRE.\textsuperscript{[27,28,117]}
Extended-Spectrum Cephalosporins:

Studies confirmed the importance of previous extended-spectrum cephalosporin treatment as risk for the VRE acquisition. Bonten et al. studied 13 ventilated patients who acquire VRE and 25 who did not acquire VRE and observed that broad-spectrum cephalosporin use predicted acquisition, whereas vancomycin use was not a significant predictor in these patients. More recently, D’Agata et al. showed that treatment with broad-spectrum cephalosporins predicted VRE acquisition among haemodialysis patients. A 52 week surveillance study of patients with hematologic malignancies substantiated the observation of an association between colonization with antibiotic resistant *E. faecium* and treatment with broad spectrum cephalosporin, which preceded the intestinal overgrowth with *E. faecium* in 93% of the patients.[118]

There is a significant association between antecedent broad spectrum cephalosporins therapy and VRE colonization or infection.[118] In a recent prospective cohort study using logistic regression, VRE colonization at the time of ICU admission was found to be associated with second and third generation cephalosporin.[27,28] The clinical importance of isolate could not be established, its isolation may be considered for as an indirect indication of excessive and prolonged use of broad spectrum antibiotics with the need review the effectiveness of the ongoing antibiotic policy.[119]

Anaerobic Agents:

However, there is also recent evidence that metronidazole may not be a microbiologically innocuous alternative to oral vancomycin for the treatment of antibiotic associated diarrhoea. Previous studies have also implicated prior exposure to antibiotics that have activity against anaerobes as an independent risk factor for colonization with VRE. The relation between exposure to highly active antianaerobic agents (e.g. metronidazole and clindamycin) and VRE colonization or infection was found to be significantly associated.[118] The use of oral or parenteral metronidazole (or other agents with significant antianaerobic activity) has noted as a risk factor for VRE bacteraemia, while others have suggested that metronidazole[31] or clindamycin[115] exposures is a risk factor for VRE acquisition.[27,28,117]
Colonization and Infection:

Infection with VRE typically follows vancomycin resistant enterococcal colonization, predominantly of the gastrointestinal tract. Frequently the colonization does not consequence in symptoms, but may last for long periods and serve as a reservoir for the transmission of VRE to other patients. A Mayo Clinic study that defined clearance as negative rectal VRE cultures on at least 3 consecutive tests obtained more than 1 week apart showed spontaneous decolonization in only 18 (34%) of 53 liver and kidney transplant recipients. Furthermore, VRE were detected on subsequent surveillance cultures from two of these previously decolonized patients. Spontaneous decolonization occurs infrequently and little progress is made in finding a pharmacological method to eliminate VRE from colonised patients. Antimicrobials like oral bacitracin, novobiocin and ramoplanin have revealed restricted success in permanently eradicating VRE from these patients.[101]

The emergence of vancomycin resistance in enterococci and the increasing incidence of high level enterococcal resistant to penicillin, ampicillin and aminoglycosides present a serious challenge for physicians treating patients with infections due to these microorganisms. Treatment options are often restricted to combinations of antimicrobials or experimental compounds with unproven efficacy. Resistance to cephalosporins and clindamycin occurs in almost all strains. Few isolates are susceptible to currently available macrolids and resistance to fluoroquinolones is now common. Once VRE have established in the hospital environment, their frequent resistance to multiple antibiotics (including erythromycin, tetracycline and rifampicin) makes it difficult to avoid further selective pressure in their favour.[27,117]

In many institutions, most patients from whom VRE recovered are colonized rather than infected. The ratio of colonized to infected patients may reach as high as 10:1 at hospitals in which perirectal or rectal swab specimens from high-risk patients were screened for VRE. In one study, 40% of organisms colonizing the gastrointestinal tract were *E. gallinarum* but no infection was caused by this species. Infections caused by VRE often involve intra-abdominal sites, urinary tract, bloodstream, surgical sites and vascular catheter sites.[27]

VRE infections tend to occur more in debilitated or seriously ill-hospitalized
patients. Mortality rates in patients with VRE bacteraemia may reach up to 60 to 70%.
Approximately half of these deaths may be because of directly to the infection. Papanicolaou et al. found VRE infection to be a important predictor of mortality in liver transplant patients. Linden et al. reported that enterococcal infection-related mortality was 46% in liver transplant recipients with VRE bacteraemia, which was significantly greater than the 25% mortality observed in patients with vancomycin-susceptible enterococcal bacteraemia but other studies revealed no significant differences in mortality, especially after controlling for factors such as age and APACHE II score. Patients with neutropenia, chronic renal failure or other serious conditions and liver transplant recipients seem to be the most likely to experience prolonged bacteraemia or to die because of VRE. However, there is no evidence that VRE are more virulent than vancomycin-susceptible strains of the same enterococcal species. Although many nosocomial enterococcal bacteraemias are polymicrobial, 80 to 90% of VRE bacteraemias are monomicrobial in some study series.\(^{27}\)

Patients who are colonized with VRE typically carry the organism in their bowel flora and may be colonized for extended periods (colonization up to 12 years has been documented). In general, patients those develop infections with VRE are among the most severely ill in the hospital. This complicates measurement of the rate of mortality associated with VRE infection that is directly attributable to vancomycin resistance. The literature on this issue is divided as to whether vancomycin resistance is an independent predictor of death among patients with enterococcal infections or perhaps more likely is a marker of severe illness.\(^{28}\)

Some studies have revealed that enterococcal bacteraemias are associated with refractory hypotension or hypotension with clinically significant hypo-perfusion and organ dysfunction. Hypotension has also accompanied *E. faecalis* bacteremic superinfection observed during the treatment of *E. faecium* infection with quinupristin-dalfopristin.\(^{27}\)

In addition to bloodstream infections, which are often catheter related,\(^{117}\) VRE were also been isolated from urine, intra-abdominal abscesses or surgical site infections and various tubes and drains relating to infections within the abdomen. VRE are occasionally isolated from pleural fluid and rarely isolated from cerebrospinal fluid; generally, this occurs following surgery or other instrumentation.\(^{27}\)
It is not always easy to assess the clinical significance of VRE in routine cultures or to differentiate colonization from infection. This is especially true in case of VRE isolates from urine specimens or when they are the part of polymicrobial infections. In some cases, attempts for treatment are not indicated. The extent to which VRE causes morbidity and mortality is often difficult to determine, because most affected patients have serious underlying diseases that cause substantial morbidity and death and because VRE have often been recovered in mixed cultures with other potential pathogens.[27]

Reservoirs:

Hospitalised patients with gastrointestinal carriage (80%) of enterococci appear to be the major reservoir, in the United States[5] and other parts of the world.[118] Traditionally, the patient’s own endogenous flora is regarded as the source of enterococci responsible for nosocomial infections or colonisations.[120] The most of the colonized patients are asymptomatic, this reservoir status is easily unnoticed unless surveillance culture specimens are obtained from patients at risk. The gastrointestinal tract is undoubtedly the major reservoir site for *E. faecium* (30%),[22] but positive clinical specimens in the absence of faecal carriage provide evidence for direct exogenous acquisition rather than gastrointestinal colonization and subsequent endogenous infection. In one study neonates had similar carriage rate for *E. faecalis* (48%) but no *E. faecium* or *E. avium* were isolated. Other parts of gastrointestinal tract, such as oropharynx and hepatobiliary tract can also harbour enterococci.[22] In one more study, 33% patients who acquired vancomycin resistant *E. faecium* had positive throat swab culture. Colonization at this site may follow contamination by staff hands during tracheostomy or endotracheal tube care. Oropharyngeal colonization may provide a source for cross-colonization, particularly if staff consider such manipulations “clean” and do not subsequently wash their hands. In another study, however, vancomycin-resistant *E. faecium* was not isolated from throat swabs of any patient known to be infected or colonized at other sites.[27] Several recent studies suggest that patient’s gastrointestinal tract is also reservoir for resistant enterococci.

Other major sites including wounds and chronic decubital ulcers may act as reservoirs for enterococci in hospitalized patients. Generally, enterococci are present in mixed culture in soft tissue wound infection, which usually have no clinical evidence of
infection. Asymptomatic women may also carry enterococci in high number in their vaginas, while more than 60% of men in hospital may carry enterococci in their perineal or meatal areas.[22]

A fascinating feature of the epidemiology of VRE is the difference between North America and Europe. The hospitalized VRE patients in the United Kingdom had little or no previous contact with medical institutions. Among the normal European people, up to 28% of community who are resident of some parts of Belgium have vancomycin resistant enterococci in their faeces.[117] This is more than 10 fold higher than the incidence of infections with these organisms. Although the main reservoir for enterococci in humans is the gastrointestinal tract, the percentage of gastrointestinal tract carriage of VRE varies widely, depending on the geographic location, the setting and the detection methods used.

Several of these patients resided in the farms where chickens and swines present in the farms were colonized with vancomycin resistant E. faecium. VRE has consequently been recovered from various animal sources in various European countries (those processed chickens, chicken carcasses, pork meat). The use of huge quantities of the avoparcin (glycopeptide) as a feed additive, for more than 15 years, in the United Kingdom and other European countries (now banned) was associated with the presence of VanA VRE in farm animals and meat products available to consumers. It was fed to broiler chickens, swine and cattle. These findings suggest that contaminated food products may serve as a reservoir from which non-hospitalized individuals can acquire VRE. People who live in farming communities in Europe were found to carry VanA VRE, in some cases same PFGE type was found in the farm animals. Despite a low prevalence of nosocomial infection, VRE have been isolated at different rates from healthy volunteers, animals and environmental sources. Notably, hospital infection rates in European countries are relatively stumpy.[27,28,121]

Preliminary results of a nationwide study of the prevalence of VRE in poultry suggest that approximately 80% of the consumer poultry at the retail level was colonized with VRE, possibly because of the unrestricted use of avoparcin in the poultry industry. Thus, the use of oral glycopeptides antibiotics in the animal production industry should be strongly discouraged. Recently, the European Community committed itself to a cautious approach and banned the use of avoparcin as feed additive in animals by 1 April 1997.[84]
In the United States, avoparcin is not a licensed feed additive for animals. The presence of VRE in the community and in animals is low. Culture surveys of a limited number of chickens in several cities have failed to detect VRE. The exact prevalence is still largely unknown. Further studies of animal-based food products are needed to determine if food item represent a community reservoir for VRE in this country. However, the opposite is true in North America, where VRE have not been isolated from environments outside the hospital, the overall incidence of infection with VRE is rather high and nosocomial outbreaks have been commonly reported. High colonization rates are found in hospitalized patients and this is a significant problem. Differences in antibiotic prescribing practices in Europe and the United States may contribute to this paradox.\textsuperscript{[27,28,121]}

Enterococci are very hardy organisms, which allow them to survive well on \textbf{Environmental surfaces}\textsuperscript{[37]} and medical equipment items in the patient’s room; frequently become contaminated with VRE and may serve as a reservoir for the organism in the hospital. Examples of items that may be contaminated include patient gowns,\textsuperscript{[33]} linen, beds, bedside rails, over bed tables, floors, doorknobs, washbasins, glucose meters, blood pressure cuffs, electronic thermometers,\textsuperscript{[22]} electrocardiogram monitors, electrocardiograph wires, intravenous fluid pumps and commodes. Widespread environmental contamination by VRE is especially likely to occur in the rooms of patients who have diarrhoea. In some studies, isolates from contaminated surfaces and from affected patients in the room have shown to represent the same strains of VRE. VRE may remain viable on such surfaces for days or weeks because the organisms are resistant to desiccation and extreme temperatures. For example, VRE may survive for 5 to 7 days on countertops and have been recovered after 24 hours or more after experimental contamination of bedrails, telephone headpieces or stethoscope diaphragms. Vancomycin resistant \textit{E. faecium} has also been isolated from a tourniquet four days after discharge of a colonized patient and from intravascular monitoring equipment after several days in storage. VRE have been recovered from the hands of personnel by some investigators. In a few instances, health care workers are found to have gastrointestinal colonization with VRE, but the epidemiologic significance of this finding is unclear.\textsuperscript{[27]}

Patients may remain colonized with VRE for weeks or months and often still colonized at the time of re-admission to the hospital. Their findings support earlier
reports of persistent VRE colonization among high-risk patients. The liver transplant patients (60%) with VRE remained colonized for 12 weeks or more. Occasionally patients remained VRE culture positive for as long as 1 year. Patients were persistently colonized with one or more VRE strain, as demonstrated by PFGE. Because constantly colonized patients may reintroduce VRE into a facility on multiple occasions, hospitals should develop means of prompt identification of such patients at the time of readmission so that they can be placed in isolation, pending repeat surveillance cultures.[27]

**Detection of Intestinal Colonization:**

The level of colonization with VRE in European community in parallels the level of colonization of animals with these resistant organisms. Several studies have reported the absence of VRE from animals and people in the community in the United States, in contrast to the high frequencies in hospitals.[84] During recent years, glycopeptide resistant enterococci (GRE), more commonly designated vancomycin resistant enterococci, have attracted much interest. They may be part of the intestinal micro-flora of humans and animals. While enterococci are easily cultivated on common laboratory media, the isolation of these organisms and more specifically, the isolation of GRE from heavily contaminated specimens such as faeces or rectal swabs may be problematic. Numerous types of commercially available and in-house-prepared selective agar and broth formulations have been used and recently, their sensitivities for the isolation of enterococci from various specimens are being compared. Although solid media are routinely used in screening studies, Enterococcosel broth[77,118] is the most sensitive medium and may be preferred over other media for routine surveillance.[121] Enterococcosel enrichment broth can detect enterococcal colonies as few as one to nine cfu/gram of stool. The rate of VRE isolation may be seriously underestimated in the absence of a broth enrichment step. Several investigators have reported, the isolation of VRE only after broth enrichment in antibiotic-free medium, suggesting that small number of organisms might be missed in selective media. This may reflect the limitations of surveillance cultures in detecting small quantities of VRE.[118]

Bile esculin azide agar with a vancomycin disk is less sensitive but could be easily and inexpensively used in any microbiology laboratory. Campylobacter agar with clindamycin required moderate preparation and was intermediate in sensitivity.[77] It
may be preferred over other media for routine surveillance.
Concentrations of vancomycin varying from 4 to 64 µg/ml are added to these selective media. Whereas a high concentration is likely to detect high-level vancomycin resistance, a concentration of 6 µg of vancomycin per ml has shown to be reliable for the detection of enterococcal strains with low-level glycopeptide resistance. Different media with different concentrations of antibiotics, and either direct plating or enrichment methods are used to detect VRE and these differences may result in differences in recovery rates. The use of a direct plating method on antibiotic-containing medium has shown to increase the isolation rate of resistant enterococci from faeces. Clare et al. have recently reported the isolation of GRE only after broth enrichment in antibiotic-free medium, suggesting that small numbers of organisms might be missed in selective media. The broth enrichment step used in his study almost doubled the number of vanA- and vanC1-carrying isolates. Since the sensitivity of the plating procedure can be estimated to be $10^3$ CFU/gm of faeces, the large proportion of isolates obtained only by broth enrichment illustrates the high frequency of carriers who excrete GRE in small numbers. A straightforward comparison of inoculation of both swabs and faecal samples both on agar plates and in enrichment broths has not been done because paired specimens are not obtained. Since the enrichment procedure involves the examination of a larger specimen volume, it is logical that it detects more carriers.\textsuperscript{[121]}

Unfortunately, none of the above-mentioned studies explored in great detail the effect of antibiotics on the exact detection threshold of VRE in different stool culturing techniques. However, Donskey et al. have recently shown that the density of VRE increases shortly after antibiotic exposure and decreases over time if no other antibiotics are given. When antibiotics were discontinued, the density of VRE in stool decreased in all 19 patients from whom samples were collected four or more weeks later. The mean interval between the discontinuation of antibiotics and the finding of undetectable levels of VRE in stool was 17 weeks (range, 6 to 20 weeks).\textsuperscript{[118]}

VRE colonisation can be identified by PCR technique on samples obtained from perineal, perirectal or rectal swabs or from stool specimens. Bacterial DNA was extracted using the automated MagNA Pure instrument. Then, the Light- Cycler instrument was used to detect vanA and vanB using a rapid real-time PCR assay. This method is more sensitive and faster (~3.5 v/s >72 hours) than culture for detecting VRE colonization. The assay detects the presence of genes associated with vancomycin
resistance in enterococci, \textit{van}A and \textit{van}B. A positive result indicates colonization but not necessarily infection with VRE.\cite{101}

Molecular testing for \textit{van}A and \textit{van}B may not always detect VRE (i.e. if the numbers of VRE organisms are very less). Furthermore, organisms other than enterococci (such as enteric anaerobes) may carry these \textit{van} genes that may lead to false-positive results. Therefore, some positive results may not really represent the presence of VRE. The frequency with which this phenomenon occurs is unknown, but it is considered to be rare. In theory, PCR testing should decrease the spread of VRE by more rapid identification and earlier isolation of colonized patients. However, the cost-effectiveness of this practice is unknown because some PCR-positive patients have minimal bacterial burden and thus present a low risk for the spread of VRE.\cite{101}

**Intestinal Decolonization of VRE:**

Once acquired, intestinal colonization by VRE can last for years, serving as a reservoir for potential infection of the colonized patient and for the spread of VRE to other patients. Although several attempts have been made to eradicate intestinal VRE carriage with enteric antibiotic agents, no regimen has been uniformly effective in eradicating VRE from the gastrointestinal tract. In a study using a mouse model in which VRE colonization was established, mice treated with a streptogramin antibiotic had recurrence of colonization seven days after the antibiotic was given.\cite{118}

In small case studies series on colonized patients, combinations of novobiocin with tetracycline or bacitracin plus doxycycline showed transient effects and failed to eradicate permanently. Only one non-controlled study suggested that oral therapy with doxycycline and bacitracin might be effective for longer periods. In another report, Dembry \textit{et al.} noted eradication of VRE colonization in two patients treated with doxycycline and rifampicin in a renal unit; rectal swabs from these patients were negative at 1 and 6 months. New approaches for achieving VRE decolonization are urgently required. For instance, administration of probiotic agents such as \textit{Bacillus coagulans} may represent a promising approach to intestinal VRE decolonization.\cite{118}

**Mode of Transmission:**

Historically, the source of enterococcal infections in patients is \textbf{endogenous}
flora.\textsuperscript{[120]} Earlier studies suggested that enterococcal infections were from hosts own
gastrointestinal tract. These conclusions were supported by epidemiological studies of urinary tract infection and bacteraemia that showed no indication of person-to-person spread. Endogenous strains gain access to the sterile sites either directly or by contaminated medical equipments resulting in nosocomial spread and colonisation with multidrug resistant strains.\textsuperscript{[38]} Carriage of enterococci in patient attending staff is implicated as one possible route of transmission\textsuperscript{[120]} like in husband and wife, who were drug addicts, developed endocarditis by similar strain. Another exogenous source noted in burn wound infection was contaminated porcine xenograft.\textsuperscript{[22]}

Since the advent of antibiograms and more sophisticated molecular typing tools, numerous studies have shown that person-to-person spread of enterococci may be a very significant mode of transmission. Zervos and colleagues used total plasmid content and high level gentamicin resistance marker, which was uncommon at that time, to show \textit{exogenous} acquisition of enterococci. Strains isolated from 10 patients in surgical intensive care unit, over a 2 months period, had identical plasmid content. The same epidemic strains were isolated from a door handle leading from intensive care unit, suggesting transient carriage on the hands of hospital personnel. In a recent outbreak of \(\beta\)-lactamase producing gentamicin resistant enterococci, the hands of two health care workers were culture positive for endemic strain. One nurse had chronically colonized gastrointestinal tract and care by this nurse was a significant risk factor of colonization for patients. There are evidences of single resistance enterococcal strains responsible for inter and intra hospital transmission.\textsuperscript{[22]}

Transmission of enterococci by health care \textbf{workers} whose hands\textsuperscript{[3,35,108]} become transiently contaminated with the organism while caring for affected patients is probably the most common mode of nosocomial transmission. These concepts are supported by the recovery of VRE, HLAR and other resistant enterococci from cultures of specimens from the patients and hands of health care workers\textsuperscript{[27]} and have been frequently isolated from environmental sources in hospital and nursing homes. In one study, the three-plasmid types found among the six isolates acquired in the SICU; two patients in adjacent bed and hands of health care workers from the same unit had identical plasmid profiles. This study again emphasizes the importance of cross infection of patients, by the contaminated hands of health care providers.\textsuperscript{[22]} Importance of these findings is difficult to assess, because the environment may simply be passively contaminated by stool or urine from infected patients.\textsuperscript{[3]}
The environmental transmission of enterococci may also occur by contaminated medical equipment, although this is much less important than transmission by the hands of personnel. Vancomycin resistant enterococci have been isolated from nearly every place and every object used in health care facilities, including various monitoring devices like stethoscopes, electronic thermometers, electrocardiographic monitors, pulse oximeters, glucose meters, blood pressure cuffs, call bells, keyboards, wall-mounted control panels. VRE are also isolated from furniture (telephones, air cushions, headboards, tables, chairs, bed rails), toilet seats, doors, floors, linens, and other medical equipment (ventilator tubing, pumps, wash bowls, automated medication dispensers, intravenous poles).[101]

Thermometers[14,117] contaminated with the outbreak strain were epidemiologically implicated in an outbreak. The high level aminoglycoside resistant enterococci have been found on environmental surface in both acute and extended care facilities. Other studies have shown environmental contamination with epidemic strains during outbreak of infection caused by enterococci resistant to β-lactam antibiotics and vancomycin.[22] The spread of VRE via bedpan washer machines has also been reported. Nosocomial transmission of VRE has been attributed to the use of fluidized microsphere beds[117] from which multiple resistant strains were recovered despite repeated attempts of decontamination or cleaning. Enterococci have been recovered from 07 to 30% of environmental cultures during several outbreaks. Since enterococci may remain stay alive for several days to weeks on dry surfaces, it seems convincing that contaminated surfaces may act as a source from which personnel may contaminate their hands or clothing. However, further studies are necessary to determine the scope to which these items contribute to the transmission of VRE.[27] It is possible that it plays an active role in person-to-person dissemination of these resistance organisms.

Controlling transmission of VRE in the health care environment has become challenging. Vancomycin resistant enterococci are capable of prolonged (>1 week) survival in the environment and can be transferred from environmental sites to staff hands.[101] Enterococci can survive on hospital fabrics (cotton and polyester) for 8 to 10 days.[122] These contaminated fabrics act as fomites in the transmission of enterococci. Frequent washing, disinfection and changing the hospital garments may play an important role in the prevention of transmission of nosocomial enterococcal infections.
Disposable cover gowns worn by personnel, who care for VRE patients, have also shown to be contaminated with the patient’s organism. Presumably, the clothing of the personnel who do not wear cover gowns may also become contaminated with VRE. However, presently there is no convincing proof that VRE are spread by contaminated cloths. There is no conclusive proof that enterococci, including VRE, are spread by the airborne route. Revival of VRE from animal sources in parts of Europe suggests that food-borne transmission may occur in certain geographic areas. However, there is conclusive proof of food-borne transmission of VRE in Europe (or other areas) is not yet available.\cite{27} In spite of the exercise of contact precautions in a latest study, health care workers in an ICU spread VRE from a contaminated to an uncontaminated part of a patient’s room.\cite{101}

**Antibiotics** may increase the likelihood of transmission of VRE by their effect on patients colonized with VRE. Most importantly, faecal incontinence or diarrhoea in VRE carriers may cause environmental contamination with VRE. VRE can be isolated from the faeces of healthy adults as well as admitted patients during vancomycin treatment. Parenteral vancomycin treatment does not eliminate all Gram-positive cocci in the oral and faecal flora and may increase the intestinal VRE load in VRE carriers. This may also make easy VRE transmission, since the number of VRE in a given clinical specimens is proportional to the ease with which VRE is transmitted to other body sites or to another patient. For instance, Beezhold *et al.* demonstrated that while all patients with VRE bacteraemia had rectal colonization with VRE, 86% and 57% also had colonization of the inguinal skin and the antecubital fossa, respectively.\cite{118}

The strains cluster according to their hosts suggests that the strains are host-specific. Such specificity was also observed in *Lactobacillus reuteri* strains by Ståhl and Molin, who reported that strains from humans or pigs could separate from strains from rats by restriction endonuclease analysis. The fact that no strains from animals found to cluster together with human strains in their study did not necessarily mean that animal-to-human spread does not occur. In their study, 48 isolates representing only a minute part of the immense number of strains occurring in nature were investigated.\cite{34}
Outbreaks of MDRE and VRE:

Enterococci responsible for nosocomial infections were susceptible to ampicillin and vancomycin, which are considered the drugs of choice for treating serious enterococcal infections. However, strains of enterococci resistant to ampicillin or vancomycin are being reported with increasing frequency. Outbreaks of VanA class vancomycin resistant *E. faecalis* or *E. faecium* have been reported in London, New York,[77] Philadelphia, Baltimore[37] and Providence. There was a sudden increase in the number of patients with infections caused by enterococci resistant to ampicillin, gentamicin and vancomycin. Isolates were also resistant to penicillin, ampicillin-sulbactum, mezlocillin, piperacillin and imipenem. These organisms were resistant to all licensed antibiotics normally used for treatment of serious enterococcal infections.[33]

Most of the studies reported outbreaks of colonization or infection due to vancomycin resistant *E. faecalis* or *E. faecium* (VanA). One of the unique features of the outbreak investigated is that it is due to multidrug resistant *E. faecium* with transferable VanB class resistant to vancomycin. Early reports describing VanB class resistance suggested that the resistance is chromosomally determined and some of the strains are self-transferable to other enterococci. The fact that transfer of vancomycin resistance may occur at the time of plasmid transfer or without acquisition of plasmid DNA suggests that transfer of vancomycin resistance in VanB strains may occur via conjugative transposons. This fact and the demonstration that rapid spread of the organism may occur among hospitalized patients; suggest that enterococci with VanB class may emerge rapidly as nosocomial pathogens.[33]

The emergence of VRE has alarmed the global infectious diseases in community for several reasons. Because of the inadequate therapeutic options for therapy of 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 vancomycin resistance highlight the difficulty of limiting the problem once it is established.
LABORATORY DIAGNOSIS\textsuperscript{[12,13]}

Specimen collection:

Various clinical samples such as urine, pus or wound swab, blood and body fluids are collected from the patients and processed for demonstration, isolation, speciation and different typing method of enterococci.

Microscopy:

Gram stained smear of direct specimen reveals Gram positive cocci, spherical or oval in shape, 0.6 - 2 µm (approximately) in size, arranged singly, pairs arranged in angles or short chains with bending.

Inoculation and incubation:

Clinical samples are inoculated on MacConkey’s agar, Trypticase soy agar, Brain heart infusion agar, blood agar or/and Cystine Lactose Electrolytes Deficient (CLED) agar plates. The media are incubated at 37°C under aerobic conditions for 24 to 48 hours and observed for the growth.

Blood (suppose to be sterile) specimens are inoculated in brain heart infusion broth or soya bean casein digest broth. These broths incubated in incubator or BACT- ALERT-3D machine, at 37°C under aerobic conditions, for 2-7 days and observed for the growth of organisms. Then suspected growths were sub-cultured on MacConkey’s agar and Blood agar plates or CLED agar for isolation of organism. Enterococci produce minute 0.5-1 mm, circular, moist, convex, deep-pink (magenta) [on MacConkey’s] with entire margins, showing either α or non-hemolytic [blood agar] colonies. Bile esculin agar or some other medium containing azide (inhibitors of Gram negative bacteria) used as selective medium for isolation of enterococci.

Identification:

Enterococci are identified based on cultural characteristics, Gram stain and
various biochemical tests such as bile esculin and arginine hydrolysis, fermentation of carbohydrates (given in flow chart).
A. Flow Chart for Identification

Arginine Deamination (hydrolysis)

+ve \[ \rightarrow \] -ve

Mannitol

+ve \[ \rightarrow \] -ve

Group II

Motility

+ve \[ \rightarrow \] -ve

Group I

Yellow Pigment

+ve \[ \rightarrow \] -ve

E. casseliflavus

E. gallinarum

E. mundtii

E. faecium

+ve

Sorbitol

+ve \[ \rightarrow \] -ve

F. necrophorum

E. solitaries

E. seriolicida

E. haemoperoxidus

E. species CDC PNS E2

Arginine (+) & Mannitol (-)

Sucrose/ Raffinose

Group III

~ 82 ~
Review of literature [Lab. diagnosis] 2015

+ ve
Growth at 45°C & in pyruvate

- ve
Trehalose/Xylose

+ ve
- ve
+ ve
- ve

E. hirae
E. dispar
Litmus milk/hippurate
E. ratti

+ ve

E. durans
E. villorum
Flow Chart for Identification[^12,14,19,49]

**Arginine (−)**

Mannitol

+ ve

- ve

Group IV

**Group I**

Arabinose

+ ve

- ve

Arabinose

- ve

+ ve

Raffinose

- ve

+ ve

Raffinose

Vagococcus species

- ve

+ ve

E.

avium

PYR/YP

E.

raffinosus

+/−ve−/+ ve

E.

pallens

E.

sacchrolytics

Yellow pigment

Growth at 45°C

**E. pseudoavium**

**E. species**

**E. gilvus**

**E. malodoratus**

CDC PNS E3

- ve

+ ve

hyppurate hydrolysis

E.

columbae

+ ve

- ve

E.

moraviensis

E.

canis

Arginine (−) ve Mannitol (−) ve

Sucrose
Sorbitol

\[ +\text{ve} \quad +\text{ve} \]

\[ -\text{ve} \quad -\text{ve} \]

\[ E. \text{asini} \]

\[ E. \text{cecorum} \]

\[ \text{Raffinose} \]

\[ -\text{ve} \quad +\text{ve} \]

\[ E. \text{sulfurosus} \]

\[ \text{BEH} \]

\[ +\text{ve} \quad -\text{ve} \]

\[ E. \text{species CDC PNS E1} \]

\[ E. \text{peniculicola} \]
**Antibiotic Susceptibility Tests:**

The performance of antimicrobial susceptibility testing is important to confirm susceptibility to choose empirical antimicrobial agents. It is important with species like enterococci that may possess acquired resistance mechanisms. The most striking feature of enterococci is the relative and absolute resistance of these organisms to a variety of antimicrobial agents used to treat various infections. Susceptibility of enterococci to commonly used antibiotics is determined by Kirby-Bauer disc diffusion technique.\[^{28,123}\]

Intrinsic resistance to penicillin, ampicillin and other β-lactams is readily detected by routine disc diffusion tests.\[^{28}\] β-lactamase production is tested by rapid Nitrocefin test.\[^{124}\] Other methods are not recommended in enterococci. Enzyme that destroys aminoglycosides like gentamicin also destroys tobramycin, netilmicin and amikacin, this high-level resistance is detected by using high concentration of gentamicin and streptomycin.\[^{125}\]

The rapid increase in the incidence of infections with vancomycin resistant enterococci (VRE) in the western hemisphere is the reason of great concern. The role of microbiology laboratories are important, as they provide accurate and timely detection of resistance in enterococci and are first line of defence to treat which reduces the spread of enterococcal infections. Until date, several studies have been done for assessing the accuracy of various antimicrobial susceptibility methods in detecting vancomycin resistance in enterococci. Since the occurrence of VRE is increasing in the United States, Europe and other parts of the world, it is crucial to optimize the laboratory’s ability to detect vancomycin resistance.\[^{123}\]

There are several methods to detect antibiotic resistance especially for VRE as follows \[^{123,126}\]

1. Disc diffusion
2. Agar screen
3. Agar dilution
4. E-test
5. MicroScan - Conventional & rapid
7. Molecular techniques - PCR Assay\[^{127,128}\] & Pulsed field gel electrophoresis.

Detection of vancomycin resistance is difficult in clinical microbiology laboratory. Disk diffusion sensitivity testing by standard 30 µg vancomycin frequently misclassifies intermediately susceptible isolates as fully susceptible. Presently MIC
determinations by broth or agar dilution or E test\textsuperscript{[26]} are the “gold standard” for determining vancomycin susceptibility but these methods are not suitable for routine use in the diagnostic laboratories.\textsuperscript{[129]} CLSI\textsuperscript{[130]} adopted criteria based on growth on BHI agar containing 6 µg/ml vancomycin within 48 hours. Nevertheless, some low resistance (VanB & VanC) enterococci strains do not grow on this medium; therefore, Hiramatsu \textit{et al.} suggested using BHI agar with 4 µg/ml vancomycin.\textsuperscript{[129]}

The disk diffusion test may fail to categorize as resistant, that those strains of enterococci having decreased susceptibility to vancomycin, i.e. those require MICs of 8 to 32, µg/ml. If the proposed breakpoints of Barry \textit{et al.} are used, only minor errors are seen; however, all strains for which MICs of vancomycin are 8 µg/ml were incorrectly categorized as susceptible by the disk diffusion test and could not be distinguished from the more susceptible strains (MICs of 0.5 to 4 µg/ml).\textsuperscript{[131]}

An agar screen containing 6 µg of vancomycin per ml was used with an inoculum of 10 ml (approximately 10\textsuperscript{6} cfu/ml) of a 0.5 McFarland standard suspension. The 30-well Vitek GPS-TA and the 45-well Vitek GPS-101 with an efficient GUI software, MicroScan conventional overnight Pos Combo type 6 panels and MicroScan Rapid Pos Combo type 1 panels with V.20.30 software (Dade International, West Sacramento, Calif.) were used, as recommended by their respective manufacturers. The CLSI breakpoints are used for interpretation of the results.\textsuperscript{[130]} A very major error is defined as an isolate that was resistant by the agar dilution method but susceptible by disc diffusion method. A major error is defined as an isolate that was susceptible by the agar dilution method but resistant disc diffusion method. Thus, lack of sensitivity of a given test has deemed to be more serious clinically than lack of specificity. A minor error is defined as a discrepancy between the results of the agar dilution method and the test method corresponding to one interpretation category. However, for the \textit{E. gallinarum} and \textit{E. casseliflavus} strains for which MICs were 8 to 16 µg/ml, both intermediate and resistant phenotype results were considered correct, since both interpretation categories correctly distinguish these \textit{vanC1-} or \textit{vanC2-} harbouring enterococci from fully susceptible strains (MIC ≤4 µg/ml). Similarly, sensitivity is defined as the ability of the test method to distinguish the \textit{vanA, vanB, vanC1} or \textit{vanC2} harbouring resistant enterococci from susceptible strains not harbouring these genes. Therefore, for strains with intermediate results with the reference agar dilution (MIC 8 to 16 µg/ml), both intermediate and resistant phenotype test results were considered
correct.[123] Optimal interpretations of MICs require knowledge of pharmacokinetics of drug in humans and information on likely success of particular drug in eradicating bacteria at various body sites.[126]

The comparative sensitivities of seven methods for the detection of \( \text{vanA} \), \( \text{vanB} \) and \( \text{vanC1/C2} \) VRE are given below. Almost all methods were 100% sensitive for the detection of \( \text{vanA} \)-mediated vancomycin resistance. However, it is important to note that for all of these \( \text{vanA} \) VRE MICs of vancomycin were \( \geq 256 \ \mu\text{g/ml} \) and these strains were detected easily. However, for \( \text{vanB} \) VRE, the sensitivity has been dropped to 93 percent, 53 and 47% with disk diffusion, the MicroScan rapid panel and Vitek GPS-TA, respectively. However, Vitek GPS-101, the MicroScan conventional panel, the agar screen and E-test were 100% sensitive for detecting \( \text{vanB} \) VRE. But for \( \text{vanC1/C2} \) VRE, E-test and the agar screen were the only methods that correctly identified all resistant strains. High mistake rates were produced by disk diffusion and automated methods. The MicroScan conventional panel able to detects only 7% of the \( \text{vanC2} \) \( \text{E. casseliflavus} \). The sensitivities of the other automated methods were around 67 to 90%.

The specificities of different methods were 96 to 100%. The \( \text{vanA} \) VRE can be detected by all methods, \( \text{vanB} \) VRE may not be routinely detected by Vitek GPS-TA and the MicroScan rapid panel, though the new Vitek GPS-101 appears to be a significant improvement. All methods except E-test and the agar screen persist to show problems in the detection of \( \text{vanC1/C2} \) VRE. The agar screen appears to be the most consistent and simple method for usual screening, if detection of \( \text{vanA} \), \( \text{vanB} \) and \( \text{vanC1/C2} \)-mediated resistance in enterococci is required. The new 45-well Vitek GPS-101 shows improved sensitivity, compared to the Vitek GPS-TA, without major loss of specificity.[123]

**Antibiotic Synergy Testing:**

Several methods are proposed to predict antibiotic synergy. Out of these only few screening tests (HLAR) are recommended for detecting synergy. Enterococci are becoming resistant to multiple drugs. Hence, study of resistance to synergism is recommended.[28] Various methods proposed to predict antibiotic synergy,

i. High disk content method
ii. Double disk diffusion method
iii. High content dilution method
iv. The checkerboard dilution method
v. The time-kill curve method
vi. E-test method
vii. Automated method and
viii. Molecular method
i. **High Disc Content Method:**

   Rosenthal and Freundlich were the first to report the use of high content disc to identify HLAR. They used disc of gentamicin (120 µg/disc) and streptomycin (300 µg/disc). They evaluated on Mueller Hinton and Trypticase Soya Agar with 5% sheep blood. They found only 3 out of 70 strains had high level resistance to gentamicin and 14 strains had high level resistance to streptomycin. Sahm and Torres used 300 µg and 200 µg for Streptomycin and 120 µg and 200 µg for aminoglycosides using MHA with 5% sheep blood to demonstrate HLAR and synergism. Streptomycin disc contained 300 µg of drug, whereas tobramycin, gentamicin, kanamycin and amikacin disks each contained 120 µg of drug. Disc diffusion and agar dilution tests have been performed by using Mueller-Hinton agar (recommended by CLSI).

   **Criteria for synergy:** Criteria for synergy is zone of inhibition of ≥ 10 mm diameter.\[125\]

ii. **Double Disk Diffusion Method:**

   The test organism is swabbed onto Mueller-Hinton agar similarly as in the Kirby-Bauer procedure. Then disc of the two antibiotics expected to react synergistically are placed on the plate. The distance between the discs is measured so that margins of the two antibiotic inhibitory zones would meet or slightly overlap. After allowing the disc to diffuse for 5 hours on the agar plate at 37°C, β-lactamase powder is dusted over the area with a sterile, dry cotton swab. The β-lactamase-dusted plate is then further incubated overnight before examination.\[133\]

   **Criteria for synergy:** Demonstration of a clear, crescent shaped area between the aminoglycoside and penicillin disks.\[133\]

iii. **High Content Dilution Method:**

   a. **Agar dilution method:**

      The method of Calderwood *et al.* has been used to detect high-level resistance to aminoglycosides. Isolates were inoculated with a Steers-Foltz replicator on a Mueller-Hinton agar plate containing 2,000 µg of aminoglycoside per ml. Plates were incubated at 35°C for 18 to 24 hours and results were recorded as growth (high-level resistance) or no growth (high-level susceptibility).\[134\]
Sahm and Torres evaluated various media like Dextrose Phosphate Agar, Brain Heart Infusion (BHI) Agar and Muller Hinton Agar (MHA) with 5% sheep blood and Trypticase Soya Agar with 5% sheep blood with different inoculum of *E. faecalis*. They got excellent results with $10^4$ and $10^6$ cfu/spot inoculum for gentamicin and streptomycin irrespective of the media used. They also obtained good results with tobramycin and kanamycin, when used to predict resistance to penicillin and amikacin synergism.\textsuperscript{[125,132]}

b. **Broth dilution method:**

Single concentration of an aminoglycoside is used in the micro dilution well. Zervos *et al.* evaluated 500 µg of gentamicin in Mueller Hinton broth. Sahm and Torres used 1 ml volume test with Dextrose phosphate broth and Mueller Hinton broth with an inoculum of $10^5$ CFU/ml.\textsuperscript{[125,132]} Both broths have shown good results.

**Criteria for synergy:** Criteria for synergy are as follows: no growth in the presence of aminoglycoside indicates potential synergy.\textsuperscript{[134]}

### Screening tests for high-level aminoglycoside resistance\textsuperscript{[130]}

<table>
<thead>
<tr>
<th>Screen test</th>
<th>Gentamicin HLAR</th>
<th>Streptomycin HLAR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medium</strong></td>
<td>BHI Broth or agar</td>
<td>BHI Broth or agar</td>
</tr>
</tbody>
</table>
| **Concentration** | 500 µg/ml | Broth: 1000 µg/ml  
                     |                      |  Agar: 2000 µg/ml |
| **Inoculum** | 0.5 McFarland turbidity | 0.5 McFarland turbidity |
| **Incubation** | 35\(^0\) C; ambient air for 24 hours | 35\(^0\) C; ambient air for 24-48 hours |
| **Results**  | Agar: >1 colony = Resistant 
               | Broth: any growth = Resistant |
| **QC strain** | *E. faecalis* ATCC\textsuperscript{®} 29212 - Susceptible and *E. faecalis* ATCC\textsuperscript{®} 51299 - Resistant |

Recommended by CLSI guidelines

Speigel compared the results with the following \textsuperscript{[39,125,132]}

i. In house micro dilution method with 500 µg of gentamicin per ml, 2000 µg of streptomycin per ml of Mueller- Hinton broth and an inoculum of $7.5 \times 10^5$ CFU/ml.

ii. Micro scan, micro dilution panel with inoculum of $10^5$ CFU/ml.

iii. An in-house tube micro-dilution method using Mueller-Hinton broth, with the same concentration and inoculum size.
iv. High content disc as discussed.
It was concluded that high content disc method, micro broth dilution method and single concentration agar plate method are currently reliable methods for detecting high level aminoglycoside resistance.\textsuperscript{[135]}

iv. **Checkerboard Dilution Method:**

The Micro dilution checkerboard (MDC) method of Bourque et al. used to predict synergy. Twofold dilutions of one drug are tested in combination with twofold dilutions of the other\textsuperscript{[134]} in Mueller-Hinton broth. An inoculum equal to a 0.5 McFarland turbidity standard was prepared from each isolate in Mueller-Hinton broth. Each microtiter well was inoculated with 100 µl of a bacterial inoculum of $5 \times 10^5$ CFU/ml and the plates incubated at 35\textdegree C for 48 hours under aerobic conditions.\textsuperscript{[136]}

**Criteria for synergy:** MDC-a reduction in MIC of both antibiotics of at least two dilution intervals (fourfold), indicated potential synergy.\textsuperscript{[134]}

v. **Time-Kill Curve (TKCs):**

A test for synergy was performed on Mueller-Hinton broth, using the growth curve method by Moellering et al. An overnight culture of the test strain was adjusted to an initial concentration of $1 \times 10^8$ to $5 \times 10^8$ colony-forming units per ml. Antibiotics were added as follows: penicillin G (10 U/ml); gentamicin either 2.5 or 5.0 µg/ml but always less than the minimum inhibitory concentration (MIC) for gentamicin. The assay tubes were incubated at 35\textdegree C in an ambient air for 24 hours. Samples were removed from each bottle at 0, 4, and 24 hours, and serial diluted (if required) and inoculated plates are incubated at 35\textdegree C in an ambient air for 18 to 24 hours. Colony counts were performed.\textsuperscript{[134,137,138]}

**Criteria for synergy:** A 100 or 10 (low) time reduction in bacterial colony count in the presence of both antibiotics as opposed to each one individually, indicated potential synergy.\textsuperscript{[134]}

vi. **E test method:**

There is little modification in E test for testing synergy. Strip contains two antibiotics, higher concentration at either end and minimum concentration at center of the same strip. The combinations of E test strips are placed on the same culture medium
in a cross formation, with a 90° angle at the intersection between the scales at the
respective MICs for same organism and the plates are incubated at 35°C for 48 hours. The MICs are interpreted at the point of intersection between the inhibition zone and the E test strip.\textsuperscript{[136]} E-test synergy screening is performed by applying two different antibiotic E-test strips to different sections of an MHA plate. The agar is marked adjacent to the previously determined MIC (1\textsuperscript{st} antibiotic) on each E-test strip. The strips are removed after incubating for 1 hour at room temperature. Using an E-test applicator, a new (2\textsuperscript{nd} antibiotic) strip is placed over the area of the previously removed 1\textsuperscript{st} antibiotic strip so that the 2\textsuperscript{nd} antibiotic MIC corresponded with the mark of the 1\textsuperscript{st} antibiotic MIC. First antibiotic strips were applied in reciprocate fashion. This established a concentration ratio of both 1 x MICs for the two antimicrobials. The resulting combination ellipses are read after 20 hours of incubation at 35°C. To evaluate the effect of the combinations, the FIC is calculated for each antibiotic in each combination.\textsuperscript{[137]}

vii. **Automated Methods:**

Gentamicin breakpoint \textit{E. faecium} of British, Europeans and American institutions have some difference. U Arslan \textit{et al.} reported that some of the strains with \textit{aac-aph} gene reported as susceptible by VITEK and PHONIX. Therefore, the concentration used by automates to detect gentamicin-resistant enterococci should be re-evaluated.\textsuperscript{[139]}

viii. **Molecular Method:**

Until recently, \textit{aac(6\textsuperscript{'})-Ie–aph (2\textsuperscript{''})-Ia} was the only reported gene associated with HLGR in enterococci. This bifunctional gene confers resistance to essentially all clinically available aminoglycosides except streptomycin thereby eliminating synergism between aminoglycosides and cell-wall-active agent such as ampicillin or vancomycin.\textsuperscript{[139,140]}

Three recently identified gentamicin-modifying genes are also associated with gentamicin resistance in enterococci and the elimination of synergy between aminoglycosides and cell wall-active agents. The \textit{aph(2\textsuperscript{''})-Ib} gene is associated with gentamicin (MIC $\geq$ 500 µg/ml) and other types of aminoglycoside resistance in \textit{Enterococcus faecium} and \textit{Escherichia coli} and appears to be linked with the \textit{aac(6\textsuperscript{'})-Im}
aminoglycoside resistance gene in both enterococci and Gram-negative bacilli. This
gene was detected in vancomycin resistant *E. faecium* isolates from hospitalized patients. The *aph(2")-Ic* gene is associated with gentamicin of MICs 128 to 512 µg/ml and the elimination of ampicillin/gentamicin synergism. The *aph(2")-Ic* gene was first described in 1997 in a veterinary isolate of *Enterococcus gallinarum* and has also been identified in human *E. faecium* and *Enterococcus faecalis* isolates. The *aph(2")-Id* gene, first described in 1998 in a human *Enterococcus casseliflavus* isolate confers high-level resistance to gentamicin but not to amikacin. This gene is detected in several vancomycin resistant *E. faecium* isolates from hospitalized patients.[140]

**PNA FISH test:**[141]

AdavnDx reported a fast test, for identifying *E. faecalis* and other enterococci from blood stream infection. Peptide nucleic acid probes fluorescence in situ hybridisation (PNA FISH) is able to identify enterococci within 90 minutes, PAN probe hybridization can be reduced up to 30 minutes. Conventional identification methods can take 3 days or longer, therefore up to 80% of VRE bacteraemia receives inappropriate therapy, leading to higher mortality and significant additional hospital cost. The 90 minutes PNA FISH protocol will enable laboratories to improve workflow flexibility. By providing faster results, laboratories will help clinicians further to improve antibiotic selection, care and outcome for patients with enterococcal blood stream infections.

**Typing Methods:**

Enterococci are a leading cause of nosocomial infections and exhibit multiple antibiotic resistances. There has been an increasing need to type and subtype isolates as a means of assisting infection control and epidemiological investigations of enterococcal outbreaks and for subtypings of enterococcal strains[41] both within and among various medical institutions. Furthermore, the evidence supporting the concept of exogenous acquisition of enterococcal infections has generated an additional need for typing the isolates. The investigation of nosocomial outbreaks along with the dissemination of enterococcal strains harbouring antimicrobial resistance markers is of major interest, particularly in the light of increasing occurrence of VRE.[12] Detailed investigation of the epidemiology of clusters of enterococcal infections or colonisations is limited by the lack of suitable typing scheme for these organisms.
A number of following phenotypic typing methods have applied to enterococci.

1. Biotyping \[12,142\]
2. Biochemical fingerprinting \[41\]
3. Antibiograms typing \[142\]
4. Serotyping \[41,142\]
5. Bacteriocin typing \[12\]
6. Bacteriophage typing \[41,142\]
7. Molecular typing methods

However, none of these provides suitable discrimination of clinical isolates. They are generally time consuming and difficult to reproduce and/or interpret. Some of these techniques frequently failed to discriminate among strains and therefore they have limited values in epidemiological studies. For this reason, there is still a need for a generally available, reliable typing scheme for both *E. faecalis* and *E. faecium*.

Recently, a number of laboratories have begun to assess methods based on analysis of genomic DNA.\[120\]

**Molecular Typing Methods:**

The introduction of various molecular techniques has substantially improved the ability to discriminate enterococcal isolates and has provided critical insights into epidemiological aspects of enterococcal infections. These are

1. DNA Restriction fragment analysis \[41\]
2. Pulsed field gel electrophoresis techniques (PFGE) \[143,144\]
3. Multi-locus enzyme electrophoresis (MLEE)
4. Contour-clamped homogenous electric field electrophoresis (CHEFE) \[120,145,146\]
5. Ribotyping \[41,49,120,144\]
6. DNA-DNA hybridization \[120\]
7. Analysis of whole/digested plasmid DNA \[145\]
8. IS 6770 probing
9. Digestion of amplified fragments of glycopeptide resistant genes
10. Vibrational spectroscopic techniques
11. Total plasmid profile analysis \[41\]
12. Non radioactive DNA probe \[147,148\]
13. PCR based typing \[41,49,120,127,128,143\]

5. Ribotyping \[41,49,120,144\]
   a) Random amplified polymorphic DNA PCR
   b) Repetitive element sequence (REP)-PCR \[149\]

Molecular methods can provide supportive evidence for diagnosis and epidemiological findings. These tools should be employed carefully after hypothesis, as their uses have been well formulated. The use of above mentioned molecular tools include introduction to new strains, dissemination of vancomycin resistant genetic elements, outbreak due to the spread of a single clone and confirmation of initial clonal
spread followed by establishment and maintenance of an endemic state. A coordinated investigation of clinical outcomes is linked with enterococcal virulence associated with enterococcal infection. [21]

**Pulse Field Gel Electrophoresis:**[143,144]

PFGE typing methods vary in their reproducibility and discriminatory ability. This method appeared to be an acceptable, preferable and superior compared to other methods.[26] but also reported to be to the others. PFGE especially the counter clamped homogenous electric field is currently the single most useful and reliable typing method, being considered as “gold standard” for the epidemiological analysis of enterococcal nosocomial VRE outbreak. In 1990, Murray et al. developed one of the first PFGE protocols for typing enterococci and their procedure is used, in its original form or with minor modifications, by numerous investigators.[41]

Genomic REA analyses the entire DNA content of a microbe by cleaving the chromosomal DNA and plasmid DNA into fragments small enough to be separate by electrophoresis on an agarose gel, producing greater number of bands than PFGE. This technique is equally sensitive as PFGE, except REA technique becomes difficult. If there are large numbers of bands, REA has also reported as the most specific method of epidemiological study.

In addition, 16 S rRNA sequencing considered as “gold standard” for identification of species of *Enterococcus*. The nucleotide sequences of the single stranded RNA species that have incorporated into the subunits of the bacterial ribosome can be used to ascertain the relatedness of the organisms, because in individual species these sequences have remained highly conserved during evolution. With the help of these unique RNA sequences, nucleic acid probe technology is developed for the direct detection of the organisms in clinical specimens.

Physico-chemical method, FT-IR (Fourier Transform Infra-Red spectroscopy) and Raman clustering,(which see the total cell composition and structure on the basis of different molecular vibrational modes) both of which are spectroscopic techniques proved to be capable of discriminating accurately up to the strain level. This technique opens the door for using physicochemical techniques as tool for epidemiological
purposes.
ANTIBIOTIC THERAPY

Treatment of enterococcal infections is complicated because these organisms often exhibit unusual pattern of susceptibility or resistance and it is necessary to use specialized techniques to demonstrate their true susceptibility in the clinical microbiology laboratory. For instance, standard methods of susceptibility testing will not predict resistance to penicillin-aminoglycoside synergism. Instead, the laboratory must test for high level aminoglycoside resistance or subject the organism for testing against antimicrobial combinations in vitro by methods like time-kill curves. Likewise, standard will also fail to demonstrate penicillin or ampicillin resistance in many β-lactamase producing strains. Finally, susceptibility testing may produce misleading results. The clinicians faced with a reporting suggesting that an organism moderately susceptible to penicillin and susceptible to vancomycin may conclude the vancomycin is better even though penicillin or ampicillin are the initial agents of choice for most enterococcal infections. The majority of infections caused by Enterococci can be cured with bacteriostatic therapy when the patient has normal host defences.

Serious enterococcal infections (e.g., bacteraemia and endocarditis) require treatment with a bactericidal combination of antibiotics that should include penicillin (e.g., ampicillin or penicillin G) to which the Enterococcus isolate is susceptible and an aminoglycoside (e.g., gentamicin or streptomycin) to which the Enterococcus isolate does not exhibit high level resistance. Vancomycin in combination with an aminoglycoside has demonstrated synergistic activity against enterococci both in vitro and in vivo, and is recommended as the drug of choice in patients with serious penicillin allergy or in the treatment of ampicillin and penicillin resistant strains of bacteria. However, intrinsic and acquired drug resistance complicates the treatment of enterococcal infections. Enterococci are becoming increasingly resistant to traditionally used antibiotic therapy. In addition to HLAR and ampicillin resistance, rapid spread of vancomycin resistance has left very restricted therapeutic alternatives.

Careful review of in vitro susceptibility data is required to treat infections caused by multidrug resistant E. faecium, the most commonly found group of VRE.
Empiric therapy for enterococcal infections should be guided by local patterns of drug resistance. It is a general rule of infectious diseases that foci of infection which are
Amenable to drainage should be drained, and infected foreign bodies, such as central venous catheters, should be removed. Treatment of infections due to VRE, especially in *E. faecium* is extremely problematic, because these organisms are resistant to multiple antibiotics.[28]

Currently there is no available antimicrobial agent that can eradicate VRE colonization, several treatment options exist for VRE infection. Most of the VRE isolates are resistant to penicillin and ampicillin; however, in unusual cases in which such agents are active, they can be useful therapeutic options. Antimicrobial susceptibility testing is recommended for verifying the activity of any agent that has been used to treat VRE.

**Penicillin Group:**

Formerly, most enterococcal soft tissue and UTIs could be treated with single drug therapy such as Penicillin, ampicillin or vancomycin. Although MIC of penicillin are approximately twice of ampicillin, adequate concentration are normally achieved. UTI can be treated with penicillin or ampicillin alone as long as infecting enterococcal strains do not produce β-lactamase.[124] Ureidopenicillin such as piperacillin or mezlocillin have broader spectrum activity and needed when mixed infections are suspected. Ticarcillin and carbenicillin are moderately active in vitro probably have little role in therapy for enterococcal infection.[1]

Penicillin, ampicillin or vancomycin with or without a synergizing aminoglycoside would be a reasonable choice in the non-allergic patient in infection such as UTI, peritonitis and wound infections caused by vancomycin resistant *E. faecalis*. These infections do not require bactericidal agent for treatment.[3] Almost all *E. faecalis* strains are moderately susceptible to ampicillin. Therefore, if vancomycin resistance emerged predominantly in *E. faecalis*, the treatment of these infections could be relatively easy. Unfortunately, vancomycin resistance has more commonly seen in *E. faecium*, which is intrinsically more resistant to penicillin as well as ampicillin.[17,27]

Penicillin or ampicillin (or another β-lactam drug with good activity against enterococci, such as piperacillin or imipenem) can be used to treat intra-abdominal infections due to *E. faecalis*. Many strains of *E. faecium* are now showing increasing resistance to the penicillins and imipenem.[150]
Chloramphenicol:

Chloramphenicol is one of the few agents that retain in vitro activity against many strains of multiple-drug-resistant *E. faecium*. Nevertheless, it is only bacteriostatic against these organisms and clinical failures of chloramphenicol have documented.[3] This agent has been used with limited or modest success in the treatment of VRE infections. Even though enterococcal isolates were resistant to numerous antimicrobial agents but they were susceptible to chloramphenicol. The specific contribution of chloramphenicol to patient outcome could not be determined, since the response rate to therapy confounded by numerous medical problems and multiple concomitant antimicrobials and interventions (e.g. drainage and debridement).[27]

Quinolones:

Ciprofloxacin and other quinolones introduced in the same period (ofloxacin, norfloxacin and enoxacin) have only modest activity against enterococci. The bactericidal effect is inoculum dependent and observed only at concentrations unattainable systemically in clinical use. Effectively, their use is limited to the treatment of urinary tract infections. However, their effectiveness for enterococcal infection (in general) is not demonstrated convincingly and increasing resistance in some centre may further decrease their attractiveness for enterococcal infection. Sparfloxacin, levofloxacin and trovafloxacin are more active than ciprofloxacin or ofloxacin against enterococci in vitro, but their activity has diminished against ciprofloxacin resistant strains, suggesting that their utility for treating infections due to multi resistant enterococci will be limited.[3]

Newer fluoroquinolone antibiotics with greater activity against Gram positive bacteria have been created and while enterococci remain among the least susceptible Gram positive bacteria (with *E. faecium* in general being less susceptible than *E. faecalis*); some compounds at 1 mg/ml or less inhibit 90% of strains. Among the new fluoroquinolones clinafloxacin is the most active agent against enterococci.[27] The new fluoroquinolones are used with varying success for enterococcal infections, but relapses have been known to occur, particularly when an infection is due to relatively resistant organisms.[124]

Tetracycline:

Some isolates of VRE are susceptible to tetracycline. Doxycycline and
minocycline are used in the treatment of VRE infections, often with other agents. The
tetracyclines have limited effectiveness against clinically important Gram positive cocci. Tetracycline derivatives like glycylcyclines have an excellent activity against enterococci including multidrug resistant strains. These compounds developed to overcome the three types of resistance mechanisms exhibited by many clinically important bacteria and to restore a broader range of activity to the tetracycline class.\footnote{27} \textbf{Tigecycline}, a broad-spectrum glycylcycline antimicrobial agent, became available in 2005. This novel tetracycline derivative has activity against aerobic and anaerobic Gram positive, Gram negative bacteria including tetracycline resistant isolates.\footnote{27}

**Macrolides:**

\textbf{Ketolides} are a newer class of macrolides derivatives with good Gram positive activity. These agents have a 2-keto structure, which appears to increase their stability in a weakly acidic environment. They act by binding to the 50S ribosomal subunit and inhibition of bacterial protein synthesis. They also penetrate well into phagocytes. Ketolides show in vitro activity against multidrug resistant Gram positive organisms, including staphylococci, enterococci and pneumococci. For vancomycin resistant \textit{E. faecium} strains, the potential spectrum of RP 59500 has found to be equal or superior to that of RU 64004.\footnote{27} \textbf{Erythromycin} has been used to treat enterococcal infection but most (80 to 90\%) enterococci in United States are now resistant to erythromycin and related macrolides.

**Nitrofurantoin:**

The majority of enterococcal isolates remain susceptible to \textbf{nitrofurantoin} and this drug has used successfully for UTI.\footnote{124} Nitrofurantoin is active against many isolates (90 to 96\%)\footnote{3} of VRE and might be an alternative for the treatment of urinary tract infections caused by VRE but does not shown useful activity in other VRE infections.\footnote{27,101} \textbf{Fosfomycin} also exhibit good in vitro activity against \textit{Enterococcus faecalis} and \textit{E. faecium} and may be useful for urinary tract infection due to these organisms,\footnote{3} but rapid emergence of resistance limits its usefulness as a single agent.\footnote{27}

**Old drugs:**

\textbf{Novobiocin} and \textbf{bacitracin} are an older DNA gyrase inhibitor with Gram positive activity. They have been used to eliminate stool carriage of VRE with
equivocal success. Clinical application of novobiocin has been abandoned due to the
in vitro data suggest that novobiocin is very active against VanA or VanB type vancomycin resistant *E. faecium* strains, even if these strains are concomitantly resistant to penicillin and ampicillin. Novobiocin plus ciprofloxacin has been found to be effective in a rabbit model of endocarditis. Novobiocin alone was not bactericidal but combination with fluoroquinolone was additive and bactericidal. The novobiocin in combination with doxycycline, used to treat VRE bacteraemia in handful patients successfully.[28] Its combination with ciprofloxacin is reported, but high relapse rates occurred after treatment. Resistance to quinolones, which is now common, would render such combinations ineffective because of the expected rapid emergence of resistance to novobiocin when used as a single agent.[27,101]

**Rifampicin:**

*Rifampicin* alone has very limited usefulness in the treatment of enterococcal infections because of its poor bactericidal activity against enterococci and because of the presence and the rapid emergence of subpopulations of resistant bacteria,[11] both in vitro and in vivo. Since rifampicin remains active against many strains of multi-resistant enterococci, it is often tested in combination with other agents.[27]

**Glycopeptides:**

*Vancomycin* alone is effective for enterococcal urinary tract infections.[124] *Teicoplanin* is another glycopeptide which is active in-vitro against most VanB type enterococci.[28] Teicoplanin alone has been used to treat the patients with enterococcal endocarditis (non- VRE) in Europe and preliminary results show success in these patients, but there have also been documented failures and relapse. There is in vivo development of teicoplanin resistance in a VanB *E. faecium* isolate. This finding has raised concern about this treatment option. [27]

*Daptomycin* is modifications of vancomycin or teicoplanin. It is cyclic lipopeptide fermentation product of *Streptomyces roseosporus*. Daptomycin became available in 2003. It acts on Gram-positive bacteria by disrupting bacterial membrane function.[101] Daptomycin (LY146032), an acidic lipopeptide and has in vitro activity against VRE. Its MICs were low with strains of all species of *Enterococcus* being inhibited by 8 µ g/ml and MICs at which 90% of isolates were inhibited (MIC<sub>90</sub>) being

~ 100 ~
recorded up to 4 µg/ml; glycopeptide resistant *E. faecium* strains were included.\textsuperscript{[27]}
Daptomycin use for the treatment of infections caused by VRE, non-Food and Drug Administration approved indication, are minimal. Daptomycin should not used to treat pneumonia because clinical trials have shown a high failure rate in this setting. In addition, resistance to daptomycin has begun to emerge.\textsuperscript{101}

Semi-synthetic glycopeptide \textbf{LY333328} is active agents against VRE, which demonstrates bactericidal as well as bacteriostatic activity against enterococci. Its mechanism of action is still unknown but thought to be similar to that of vancomycin. The primary mechanism appears to be the inhibition of cell wall synthesis and assembly with the D-alanyl–D-alanine precursor. It may damage RNA synthesis of enterococci. Several studies have shown that LY 333328 exhibits bactericidal activity against enterococci. The reported MIC\textsubscript{90} for different strains of MRSA and VRE (VanA and VanB strains) are ≤1 µg/ml. LY333328 has increased activity against vancomycin resistant Gram positive microorganisms.\textsuperscript{84} The excellent inhibitory and bactericidal activity of LY333328 suggest that it could be a clinically useful alternative for the treatment of severe infections caused by Gram-positive pathogens (including VRE), particularly those resistant or not fully susceptible to the available glycopeptides.\textsuperscript{127}

Other derivatives of vancomycin may show increased activity against enterococci, including glycopeptide resistant strains, but development of these compounds is at an early stage. There are also derivatives of teicoplanin, LY264826 and other glycopeptides with increased anti-enterococcal activity. Some of them are not bactericidal, some show cross-resistance with teicoplanin- and vancomycin resistant strains, and they are still at early stages of development. It is clear that before this group of antimicrobial agents can offer new treatment possibilities for these infections, further work will be needed to produce a satisfactory bactericidal glycopeptide that is effective and nontoxic in systemic use and lacks cross-resistance with vancomycin and teicoplanin.\textsuperscript{127}

\textbf{Ramoplanin:}

Ramoplanin (lipoglycodepsipeptide) is even more active. In investigations conducted, all the species of enterococci including vancomycin resistant \textit{E. faecalis} and \textit{E. faecium}, were inhibited by 8 µg/ml and the MIC\textsubscript{90} ranged from ≤ 0.25 to 1.6 µg/ml. Ramoplanin is also bactericidal for enterococci, with the MBCs being only fourfold
higher than the MICs. It inhibits cell wall synthesis by acting at the level of lipid-
intermediate formation, whereas vancomycin and daptomycin interfere with
peptidoglycan synthesis by preventing transglycosylation. Addition of human serum
results in fourfold increase in the MIC. In preliminary studies, ramoplanin is poorly
tolerated following intravenous or intramuscular injection and it seems unsuitable for
systemic use because of its toxicity; however, it has developed for topical use. It is
suggested that ramoplanin used for the clearance of glycopeptide resistant enterococci
from the gastrointestinal tract.\[27\]

**Quinupristin-Dalfopristin:**

Quinupristin-dalfopristin (pritinumycin) used for treatment of multidrug
resistant enterococci (MDRE) which are resistant to penicillin, aminoglycosides and
glycopeptides. It is a parenteral combination of two streptogramins antibiotic with good
individual bacteriostatic activity, but combination is often bactericidal, is more potent
and may be active even when there is resistance to one component.\[151\] It has been
studied in the treatment of infections due to vancomycin resistant \textit{E. faecium} but has
poor activity against \textit{E. faecaluis}.\[28\]

In late 1999, quinupristin-dalfopristin became the first antimicrobial agent
available for the treatment of vancomycin resistant \textit{Enterococcus faecium} infection.
Although, \textit{E. faecium} (the most common vancomycin resistant \textit{Enterococcus} species) is
susceptible to quinupristin-dalfopristin, most of \textit{Enterococcus faecalis} isolates and
many other non \textit{E. faecalis} - non \textit{E. faecium} species are intrinsically resistant to this
antimicrobial agent. Quinupristin-dalfopristin, a streptogramin targets the bacterial 50S
ribosome, thereby inhibiting protein synthesis.\[151\] The most common adverse effects
are arthralgias, myalgias.\[101\] Relatively few clinical isolates of \textit{E. faecium} are resistant
to quinupristin-dalfopristin (5% of initially). However, this figure increased (up to
86%) when the survey also included subsequent patient isolates, indicating that
resistance may develop in a minority of patients while on therapy.\[28\]

It does not exhibit synergy with any existing antibiotics. Invitro studies have
demonstrated increased activity of RP 59500 when combined with vancomycin against
MRSA. However, no synergy has found against VRE when the drug combined with
ampicillin, ciprofloxacin, gentamicin, rifampicin, streptomycin, teicoplanin, or
vancomycin. RP 59500 was first made available and approved for treatment of patients
with only serious or life threatening infection like bacteraemia due to vancomycin resistant *E. faecalis* and *faecium*.\[27]\]

In a recent study by Linden *et al.* had compared the clinical and bacteriological outcomes of 20 patients with VREF bacteraemia treated with RP 59500 with a historical cohort of 42 patients with VRE bacteraemia treated with other agents. They found that in spite of the high mortality rates in both groups, quinupristin-dalfopristin therapy was associated with a significantly lower incidence of VREF associated mortality. On the other hand, frank clinical failure has seen in five quinupristin-dalfopristin treated patients. One failure occurred in a patient with refractory neutropenia following drug induced bone marrow suppression. The lack of bactericidal activity of quinupristin-dalfopristin may compromise its use in treatments; where bactericidal activity is required for eradication of enterococci from severe infections.\[27]\]

The satisfactory outcomes are reported in other challenging clinical conditions. Quinupristin-dalfopristin therapy achieved microbiological and clinical cure in a patient with vancomycin resistant *E. faecium* prosthetic valve endocarditis, in an eight months old infant with ventriculitis due to a vancomycin-resistant *E. faecium* infected central nervous system shunt and in three cases of vancomycin resistant *E. faecium* peritonitis due to peritoneal dialysis catheter associated infection. Superinfection with *E. faecalis* was observed during therapy of *E. faecium* infection with this agent. De Novo reported that resistance to quinupristin-dalfopristin among *E. faecium* strains and the rise in MICs of quinupristin-dalfopristin to 1 to 2 µg/ml raises the possibility that frank resistance to quinupristin-dalfopristin could develop in some strains. An increase in the MIC of the drug associated with relapse after therapy has also observed.\[27]\] The criteria of the CLSI for susceptibility, intermediate susceptibility and resistance to quinupristin-dalfopristin are \( \leq 1, 2 \) and \( \geq 4 \) µg/ml, respectively.\[151]\]

**Oxazolidinone:**

The oxazolidinone are a new class of synthetic antibiotics with good anti-enterococcal activity and are different from any other class. The mechanism of activity include binding to the ribosome and inhibiting protein synthesis\[151]\] leading to a bacteriostatic effect against most species. The oxazolidinones inhibit bacterial translocation at the initiation of protein synthesis.\[27]\] They are active when given orally. Eperzolid and linezolid are two investigational oxazolidinone agents. They show
excellent activity against multiple antibiotic resistant enterococci characterized by low MICs. Clinical efficacy and safety studies are essential to determine their definitive effectiveness.[27]

**Linezolid** is the first new class of antimicrobial agents termed oxazolidinones, became available in 2000. It can be given orally or intravenously and unlike quinupristin-dalfopristin. It has activity against both *E. faecium* as well as non–*E. faecium* species also like *E. faecalis*, *E. casseliflavus*, *E. gallinarum*. [151] Recently, linezolid is the only oral agent approved by the Food and Drug Administration (FDA) in the United States and Europe as well as other countries for treatment of infections caused by VRE. The most recently approved drug with good activity against enterococci and staphylococci is linezolid. They interfere with bacterial protein synthesis by binding to domain V of ribosomal RNA, which is encoded by genes (ribosomal RNA gene) present in multiple copies.[101] They also binds to the 23S rRNA of the 50S subunit on the bacterial ribosome.[27,28,151,152] Linezolid inhibits ribosomal protein synthesis but at a different site from other agents that target the ribosome (chloramphenicol, macrolides, lincosamides, streptogramin, aminoglycosides, tetracycline).[101]

Linezolid has nearly uniform activity against enterococci and with MICs of 1-4 µg/ml in one study including 180 isolates of various enterococcal species, regardless of vancomycin susceptibility.[27,28] Linezolid is the anti-VRE drug used most commonly at the various clinics. However, its myelo-suppressive adverse effects, especially thrombocytopenia, may limit its use in some patients.[101] Duration of therapy has also shown to be the major risk factor for bone marrow suppression due to linezolid use.[152] Linezolid is a weak monoamine oxidase inhibitor, a diet low in tyramine is generally recommended while taking the medication. Linezolid has some potentially important drug-drug interactions and careful review of the patient’s medical regimen, in consultation with a pharmacist, has recommended before it is prescribed.[101]

Unfortunately, linezolid resistant enterococci have emerged and spread nosocomially. In 2001, for example, seven clinical isolates of linezolid resistant, vancomycin resistant *E. faecium* were reported from the Mayo Clinic.[101] Linezolid is used primarily to treat serious VREF infection: 80% of treated patients received therapies in an intensive care unit most have multiple co-morbidities. These organisms
may transmit from patient to patient in the hospital: nosocomial spread of linezolid resistant, vancomycin resistant *E. faecium* (LR-VREF) in a transplant unit has recently described.\(^{[152]}\) The LR-VREF strain was identified in a liver transplant recipient whose course was complicated by vancomycin resistant *E. faecium* intra-abdominal infection treated with linezolid therapy. The strain was transmitted nosocomially to six other patients despite strict isolation of the index case, the use of private rooms and universal gloving by health care workers before entering patients’ rooms.\(^{[101]}\)

Linezolid resistant isolates have remained susceptible to quinupristin+dalfopristin, so this agent may be a reasonable alternative therapy for patients infected with LRVREF. However, emergence of in vitro resistance to quinupristin-dalfopristin among VREF has also been observed in several prospective studies; thus, performing susceptibility testing for all isolates recovered from patients while they are receiving antimicrobial therapy for serious infections due to Gram positive organisms.\(^{[152]}\) Initial clinical trials have shown that the linezolid has good efficacy with fewer adverse reactions. Although enterococcal resistance to this drug have begun & till it is rare.\(^{[28]}\)

Given the difficulty of treating VRE when they have recovered from infected sites and the fact that enterococci are not highly virulent, the question sometimes arises: Do we really need to treat the patient with antibiotics active against VRE? Certainly yes, infective endocarditis, urinary tract infections and any infection of a sterile space with VRE should treat aggressively. Patients with VRE infective endocarditis may benefit from early valve removal. VRE bacteraemia related to IV catheters may resolve spontaneously after removal of the infected catheter. However, treatment with the best available drug for any VRE bacteraemia have probably warranted and highly recommended for patients with prosthetic or otherwise abnormal heart valves in an attempt to prevent endocarditis. Linezolid and quinupristin-dalfopristin are most likely to be active (the latter against *E. faecium* only), but tetracycline drugs and chloramphenicol may be considered as well. Treatment has clearly indicated for VRE bacteraemia related to abdominal infections or complicated soft-tissue infections.\(^{[28]}\)

The role of enterococci, including VRE, in polymicrobial skin and soft-tissue infections has debated. Infections at sites such as surgical wounds, decubitus ulcers and diabetic foot those involve usually mixed flora including VRE may disappear without specific treatment for VRE when the more virulent pathogens are effectively treated.
However, the overall clinical picture should be considered, and treatment directed against VRE is indicated in some cases. VanC enterococci (E. gallinarum and E. casseliflavus) are relatively uncommon pathogens. They are naturally sensitive to penicillins and other routinely used drugs and therefore they treated easily.[28]

**Future options** for treatment of VRE infection may include mannopeptimycins and dalbavancin. Mannopeptimycins, a novel class of glycopeptides, are semisynthetic antimicrobials derived from *Streptomyces hygroscopicus*. They inhibit transglycosylation of cell wall peptidoglycans. They are active against a wide variety of Gram positive bacteria, including VRE, and have shown to be bactericidal in vivo. Dalbavancin has gained attention because of its once in week dose. However, it is active against VanB but it has little activity against the most common VanA glycopeptide resistant phenotype enterococci.[101]

**ANTIBIOTIC COMBINATION THERAPY:**

The combination of cell wall active agents (usually penicillin, ampicillin or vancomycin) with aminoglycosides (usually streptomycin, gentamicin or other) has been standard for treatment of enterococcal endocarditis since the first demonstration of penicillin and streptomycin synergy in 1947. Relapse rates (30 to 60%) are demonstrated in patients with enterococcal endocarditis due to organism with high-level gentamicin resistance and treated with penicillin or ampicillin alone. Now a days, generally Penicillin or ampicillin plus gentamicin or streptomycin are used for enterococcal endocarditis and meningitis.[1,3]

Vancomycin is substitute for penicillin or ampicillin in combination with gentamicin or streptomycin in patients who are allergic to penicillin. In most of the cases, 4 weeks of combination therapy given to the patient appears to be adequate, with 6 weeks regimens given to the patients who have symptoms more than 3 months before starting the treatment, patient with prostatic valves or patients who have relapsed after previous shorter courses of therapy. Similar therapeutic regimens are used to treat patients with enterococcal meningitis. But there is no sufficient data available with this disease to assess the therapeutic effectiveness or optimal length of treatment. Most of the patients with enterococcal meningitis seem to respond to treatment, which is generally given for 2 to 3 weeks.[3]
The possible exception would be the strains containing phosphotransferase, which mediate high-level resistance to gentamicin without accompanying acetyltransferase activity. Some of these strains are susceptible to cell wall active agents and amikacin but sufficient data are not available regarding this combination. Intravenous ampicillin (which produces greater killing of enterococci in vitro than penicillin alone) for longer periods (8 to 12 weeks) is recommended to treat such patient. There are not enough data to know how effective such therapy will be, but there are examples of success.\(^3\)

Routine testing by the clinical laboratory will classify \textit{E. faecium} strain as ampicillin resistant if it is able to grow in of ampicillin 16 µg/ml.\(^3\) Higher concentrations are not routinely tested. However, if the organism is inhibited by ampicillin (32 µg/ml), then it use ampicillin, since it should be possible to exceed this concentration several fold in vivo with high doses. Therefore in case of \textit{E. faecium} infections resistance to ampicillin and vancomycin, the laboratory should perform further susceptibility tests to determine the MIC of ampicillin. Strains for which the ampicillin MICs are ≥64 µg/ml\(^{28}\) are now common and this concentration (>100 µg/ml) is close to the limit of concentrations achievable in the serum.\(^{27}\) Mekonen \textit{et al.} described the failure of ampicillin at a total dose of 20 g/day (mean level in serum, 10^3 µg/ml) combined with gentamicin\(^{17,117}\) to clear VRE bacteraemia in a liver transplant patient. Ampicillin-gentamicin combination has shown to be highly efficacious for the treatment of enterococcal infections. Although enterococci some time show susceptibility to this combination in laboratories but during therapy it may not be that much effective due to production of aminoglycoside modifying enzymes that may have been increased in these strains. Because of increase in gentamicin resistance and renal toxicity, alternate combination that include agent such as ceftazidime, amikacin and vancomycin might have been recommended for the initial treatment of encephalitis and other infections.\(^{17}\) Many VRE (\textit{E. faecalis}) remain relatively susceptible to penicillin and ampicillin (MICs of 0.5 to 2 µg/ml), and these agent can be tried therapeutically for infection caused by this organism.\(^3\)

Substitution of ampicillin-sulbactum at 30 gm/day (equivalent to 20 gm of ampicillin; mean ampicillin level in serum, 130 µg/ml) led to clearing of the bacteraemia. The authors attributed this success to the slightly better activity of ampicillin combined with sulbactum compared with that of ampicillin alone against the
A clinical isolate of *E. faecium* (MIC, 32 and 64 µg/ml, respectively). It has been known that some strains are approximately one dilution more susceptible to ampicillin when tested in the combination. If the infecting VRE (*E. faecium*) is highly resistant to ampicillin or penicillin has shown to produce bacteriostatic but not bactericidal synergism against some of such organism in vitro. However, this has not been universally true and although combinations of ampicillin plus vancomycin plus gentamicin have demonstrated bactericidal activity against several enterococcal strains in animal models; the clinical effectiveness of such therapy remains undemonstrated.

Despite the fact that vancomycin resistant enterococci of the type VanB phenotype remain susceptible to teicoplanin in vitro, but it has not been universally successful; because there may be emergence of resistance to teicoplanin during therapy. The cases of enterococcal endocarditis due to VRE (VanB phenotype) were successfully treated with teicoplanin plus aminoglycoside. Combination therapy seems essential to prevent the emergence of resistance.

### Antimicrobial Therapy of Enterococcal Endocarditis

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Route</th>
<th>Dosage</th>
<th>Duration(wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organisms not highly resistance to streptomycin or gentamicin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin G OR</td>
<td>IV</td>
<td>20 - 30 million U / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td>Ampicillin plus</td>
<td>IV</td>
<td>12 – 16 g / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td>Streptomycin OR</td>
<td>IM</td>
<td>20 mg / kg / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>IM / IV</td>
<td>3 – 5 mg / kg / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td><strong>Organisms highly resistance to streptomycin but not gentamicin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin G OR</td>
<td>IV</td>
<td>20 - 30 million U / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td>Ampicillin plus</td>
<td>IV</td>
<td>12 – 16 g / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>IM / IV</td>
<td>3 – 5 mg / kg / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td><strong>Patient allergic to penicillin; desensitization not feasible</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin plus</td>
<td>IV</td>
<td>30 mg / kg / d</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td>Streptomycin OR</td>
<td>IM / IV</td>
<td>Use above guidelines in choice of aminoglycoside</td>
<td>4 - 6 weeks</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Organisms highly resistance to streptomycin and gentamicin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>IV</td>
<td>12 – 16 g / d</td>
<td>8 - 12 weeks</td>
</tr>
</tbody>
</table>
When enterococci have high level resistance to both gentamicin and streptomycin, no regimen currently available was likely to produce a reliable bactericidal effect. There were few unpredictable reports with high-dose ampicillin alone, vancomycin, teicoplanin, or ciprofloxacin, either alone or in combination with a penicillin or daptomycin. Currently, it is not known what constitutes will be the best therapy for enterococcal endocarditis due to strains with high level resistance to all aminoglycosides. A reasonable approach would be continuous high dose of ampicillin, probably for longer than the usual 6 weeks\(^{[27]}\) that may be for 8 to 12 weeks.\(^{[1]}\)

The optimal therapy for patient with serious infections caused by enterococcal strains that show high level resistance to all aminoglycosides known, but adding an aminoglycoside to cell wall active agent adds nothing to the therapy except for toxicity. No clinical data suggest that any single drug regimen reliably produce cure. However, animal model studies with one strain showed that continuous infusion of ampicillin was more effective than intermittent ampicillin therapy for enterococcal endocarditis caused by single strain that was highly resistant to gentamicin.\(^{[3]}\)

\(\beta\)-Lactamase producing enterococci remain susceptible to vancomycin (and teicoplanin) and to combination of \(\beta\)-lactam and \(\beta\)-lactamase inhibitors such as ampicillin-sulbactam and amoxicillin-clavulananate. These agents have shown to be effective in animal models as well. Nevertheless, there are no convincing published reports of endocarditis due to \(\beta\)-lactamase producing organisms, so the clinical effectiveness of these regimens cannot be assessed.\(^{[3]}\)

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 explored in experimental animal models. Each approach has limitations. The combination of a glycopeptides and a \(\beta\)-lactam is useful for treatment of VRE patients although some strains of *E. faecium* resistant to ampicillin and vancomycin individually, have still inhibited by the combination of these two antibiotics. For such strains, the MIC of ampicillin decreases in the presence of vancomycin. This is possibly because the cell, in order to use the vancomycin-induced D-Ala–D-Lac-containing precursor, must shift to using a different cell wall synthesis enzyme.\(^{[28]}\) If this enzyme is more easily inhibited by ampicillin, the MIC of ampicillin would decrease. The laboratory can predict which strains will show this interaction by comparing the results of
ampicillin disk susceptibility using agar plates with and without vancomycin. Unfortunately, many ampicillin-resistant, vancomycin-resistant *E. faecium* strains do not show this phenomenon even with those that do, subpopulations resistant to the combination usually exist and can preferentially selected after exposure to the combination.[27]

The combination of **ceftriaxone, vancomycin and gentamicin** are reported to be significantly more effective than either penicillin-vancomycin-gentamicin or penicillin-teicoplanin-gentamicin in the treatment of experimental penicillin- and glycopeptide-resistant *E. faecium* endocarditis. Combinations involving double β-lactams have also examined, and the combination of ampicillin and imipenem appeared to have a positive interaction in an experimental model of endocarditis in rabbits.[27]

There are reports of in vitro activity of other agents including novobiocin, ciprofloxacin, fosfomycin and pristinomycin in various combinations regimen against enterococci, but none of these have been demonstrated clinically useful.[3] The combination of ampicillin at 20 µg/ml with clinafloxacin at 1 µg/ml also had bactericidal activity against enterococcal strains when the drugs were present in serum at concentrations that are easily attainable.[27] The treatment of infections due to multiple drug resistance enterococci remains a highly empirical endeavour and requires the backup of a clinical or research laboratory well versed in antimicrobial susceptibility testing.[3] Other treatment barriers to address are resolution of neutropenia, reduction in antibiotic pressure, drained closed space infection and removal of foreign bodies.[1]

In a rat model of endocarditis, synergy with ciprofloxacin plus either gentamicin or rifampicin or both have shown in vitro and in vivo activity against strains of vancomycin resistant *E. faecium*. Triple therapy (ciprofloxacin-rifampicin-gentamicin) was most effective at sterilizing vegetations. Although ciprofloxacin in high persistent concentrations may be effective in treating enterococcal endocarditis in the rat model but concentrations attainable in serum in humans have not yielded satisfactory results. In time-kill curve studies using strains of *E. faecium* with high-level resistance to ampicillin, vancomycin and aminoglycosides, the combination of ampicillin at 40 µg/ml plus ciprofloxacin at 3 µg/ml was bactericidal for all strains with ciprofloxacin MIC of 8 µg/ml or below.[27]
EMERGENCE OF ANTIMICROBIAL RESISTANCE

Even though, enterococci are commensals in adult faeces, they are important nosocomial pathogens. Their emergence in a past three decades is attributable to their resistance to many commonly used antibiotic agents. Enterococci have remarkable ability to survive in an environment of heavy antibiotics.[32] Imprudent uses of antibiotics and colonisation pressure is the important cause of the drug resistance.[153]

Antimicrobial resistance can be either intrinsic or acquired:[25,27,28,32]

Types of resistance and different examples of antimicrobial drugs

<table>
<thead>
<tr>
<th>Intrinsic</th>
<th>Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactams (penicillinase susceptible penicillins (low level), penicillinase resistant penicillins &amp; cephalosporins)</td>
<td>High concentrations of β-lactams (via penicillin-binding proteins or β -lactamase)</td>
</tr>
<tr>
<td>Low concentrations of aminoglycosides</td>
<td>Glycopeptides (vancomycin &amp; teicoplanin)</td>
</tr>
<tr>
<td>Clindamycin (low level)</td>
<td>High concentrations of aminoglycosides</td>
</tr>
<tr>
<td>Fluoroquinolones,</td>
<td>Clindamycin (high level)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole (in vivo)</td>
<td>Fluoroquinolones,</td>
</tr>
<tr>
<td>Lincosamide,</td>
<td>Tetracycline, Erythromycin,</td>
</tr>
<tr>
<td>Nalidixic acid,</td>
<td>Rifampicin, Chloramphenicol,</td>
</tr>
<tr>
<td></td>
<td>Fusidic acid, Nitrofurantoin,</td>
</tr>
</tbody>
</table>

They have a remarkable ability to adapt to exposure to the antibacterial agent maintaining intrinsic resistance to penicillins and low level resistance to aminoglycosides and a tremendous capacity to acquire resistance to other antibacterials including high level resistance to aminoglycosides and glycopeptides.[154]

INTRINSIC OR INHERENT PROPERTY

The all or most strains of species are resistant to particular antibiotic are known as intrinsic resistance. The genes for intrinsic resistance appear to reside on chromosomes.[115,109] The various intrinsic traits expressed by enterococci are follows.[20,29]

- Resistance to β-lactams, semisynthetic penicillinase resistant penicillins and cephalosporins.
- Resistance to low levels of Aminoglycosides
- Resistance to low levels of clindamycin, trimethoprim, sulfamethoxazole and
fluroquinolones.\textsuperscript{[20]}
a) β-Lactams:

Complete or relative β-lactams resistance are the characteristics of the genus Enterococcus. E. faecalis is typically 10 to 100 times less susceptible to penicillin than streptococci. While, E. faecium is at least 04 to16 times less susceptible than E. faecalis. The major mechanism underlying this resistance is the production of low affinity penicillin binding proteins (PBP), which is useful for them to synthesize cell wall components even in the presence of modest concentration of most β-lactam antibiotics. Most of the isolates of E. faecalis can be inhibited by concentration of penicillin achievable in the plasma (MIC of 1 to 8 µgm/ml).

b) Aminoglycoside Resistance:

Enterococcal resistance to gentamicin and streptomycin occur by different mechanisms, it is important to test susceptibilities to both agents. Low level (MIC 8 to 256 µgm/ ml) and moderate level (MIC 62-500 µg/ml) aminoglycoside resistance is due to decreased cell wall permeability. However, aminoglycoside uptake is enhanced when enterococci exposed to β-lactams. These synergies with both classes of antibiotics used to treat the serious enterococcal infections. These combinations overcome the intrinsic resistance exhibited by enterococci and cell wall active agent facilitates a synergistic effect usually achieved since intracellular penetration of aminoglycoside.

c) Other Antibiotic:

Another important feature of enterococci is their clindamycin and lincomycin resistance. MICs for most strains are 12.5 to 100 µg/ml. Low level resistance was reported by Moellering and Krogstad. The combination therapy of sulfamethoxazole and trimethoprim may appear to be active against enterococci invitro, the microorganisms are presumed to be clinically resistant by virtue of their ability to use exogenous folate, thus circumventing the mechanism of action of those drugs.

ACQUIRED RESISTANCE

This resistance results from a mutation either in existing or acquisition of new DNA is known as acquired resistance. Enterococci had acquired resistances to followings.

- High concentration of β-lactams: through penicillin binding proteins or β-lactamase.
- High level clindamycin, aminoglycoside, glycopeptides (vancomycin) and lipopeptide (teicoplanin).
Enterococci with higher penicillin resistance with MIC >256 µg/ml are now becoming more common. The increased MICs of Penicillin for these strains may be because of similar but extreme intrinsic resistance (low affinity PBP or acquired resistance). The enterococci is intrinsically resistance to commonly used antimicrobial agents may help them for further acquisition of genes encoding high level resistance to aminoglycosides, penicillins, tetracycline, chloramphenicol and now vancomycin also. There are at least three major reasons for the emergence of multidrug resistant enterococci: (i) baseline intrinsic resistance to several antimicrobial agents, (ii) acquired resistance via mobility of the resistance genes on plasmids and transposons, and chromosomal exchange and (iii) the transferability of resistance.[27] In addition, these genetic transfers often occur in the gastrointestinal tracts of humans and animals. Many of which have other bacteria under potential selective pressure from therapeutic or subtherapeutic levels of on-going antimicrobial exposure. Finally, the environmental burden of antimicrobial utilization, colonization pressure and nosocomial transmission of VRE is high in many hospitals and may be high in the animal health industry, where the organisms appear to be hearty survivors.[25]

**a) β-Lactam Resistance**

High-level resistance to β-lactams has dramatically increased during the last two decade. The mechanism of this resistance results from low affinity penicillin binding protein or as result of transferable β-lactamase property from staphylococci.[16]

**Tolerance**

The enterococci are “tolerant” to activity of β-Lactams, that is, enterococci are inhibited but are not killed by these agents. This property is an acquired characteristic. After exposure to as few as five doses of penicillin, enterococci may quickly develop a tolerance. Most of the enterococci are tolerant (i.e., MBC/MIC of >32 µg/ml)[27] to cell wall active agents like penicillins or glycopeptides. However, they may be killed by synergistic effect of penicillin / ampicillin with aminoglycoside: standard treatment for serious infection.[32] The ability of the organism to survive levels of drugs well in excess of the MIC is tolerance. Tolerance to cell wall-active drugs (e.g. penicillin and vancomycin) is common among clinical isolates of enterococci and thought; they are intrinsic until non tolerant isolates of *E. faecalis*, are obtaining from an antimicrobial drug-naive population. It is subsequently demonstrated that these strains could become tolerant by exposure to pulsed doses of penicillin.[28]
**β-Lactamase:**

β-Lactamase producing enterococci reported for first time in 1983, is still an uncommon property of these organisms. β-Lactamase production is encoded by transferable plasmid. This gene is highly homologous to the gene for β-lactamase of staphylococci. This strongly suggests the acquisition of gene from staphylococci. β-Lactamase from Staphylococci is inducible and freely secreted into the medium. Unlike staphylococci, β-lactamase by enterococci is constitutively producing (cell bound), low level and inoculum dependent. Treating patient with enterococcal infections has become difficult. The enzyme hydrolyses penicillin, ampicillin and piperacillin, that is the mainstay of therapy for enterococcal infection. Development of resistance by enterococci to this drug has important clinical implication. Actually, there are many penicillin resistance reports available from west, but we get comparatively less number of such reports.

β-Lactamase production is plasmid mediated. Because amount of β-lactamase production by enterococci is insufficient for detection of routine antibiotic susceptibility testing, so isolate from serious infections should be screened specifically for β-lactamase production. Recommended and reliable method for β-lactamase production is only chromogenic cephalosporin (nitrocefin). E. faecalis (not E. faecium) strains producing β-lactamase are not susceptible to anti-staphylococcal penicillins but are susceptible to ampicillin, amoxicillin and piperacillin combined with β-lactamase inhibitors such as sulbactum, clavulanic acid and tazobactum respectively.

**Penicillin Resistance without β-Lactamase:**

High level resistance to penicillin (HLPR) and ampicillin have been reported from various places. HLPR is defined as MIC of penicillin is 256 µg/ml or more. This drug concentration is essentially unachievable in the serum for any significant duration of time. The MIC of ampicillin is one dilution lower i.e. 100 µg/ml. Penicillin resistance is directly proportional to the amount of PBP-5 (a specific PBP) produced. High level penicillin resistance in E. faecium (MIC Fontana et al.) showed that loss of the ability of a strain of E. faecium to produce PBP-5 caused this highly penicillin resistant strain to become hyper susceptible to penicillin. β-lactamase producing enterococci are infrequently isolated. Most of the isolates of E. faecalis are inhibited by concentration of penicillin / ampicillin 1-8 µg/ml easily achievable in...
While, isolates of *E. faecium* require more concentration, usually an average of 16 to 64 µg/ml to inhibit the growth, although some isolates are even more resistant. Concentration of ampicillin that are needed to inhibit the enterococci are about half of the penicillin. Valerie AC reported penicillin and ampicillin resistant *E. raffinosus*. 

### b) Other Antibiotics

Other common acquired genetic determinants confer resistant to fluoroquinolone, macrolides, tetracycline and chloramphenicol. Chromosomal mutations may occur that produce resistance to rifampicin and fusidic acid among others. Vancomycin is the most important antibiotic for the treatment of enterococcal infections.

The enterococci survive in the hospital environment because of their intrinsic resistance to several commonly used antibiotics and perhaps more important, 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.

### c) Glycopeptides:

Vancomycin resistance has detected most routinely in ampicillin resistant *E. faecium* isolates. There is an association between ampicillin and vancomycin resistance. The clinical studies have shown that prior β-lactams (cephalosporins) use are a leading predisposing factor to contribute to the emergence of vancomycin resistant *E. faecium*. The sporadic detection of vancomycin resistant *E. faecalis* is due to the linkage between a β-lactam resistant (penicillin binding protein) and vancomycin resistance does not yet appear in *E. faecalis*. 

The clonal dissemination of certain enterococcal strains, increased environmental enterococcal burden secondary to antimicrobial regimens and limitations of effective infection control measures have together contributed to the rising endemicity and nosocomial outbreaks from VRE. It appears that many clones of *E. faecium* and *E. faecalis* never proliferate and the majority of *E. gallinarum* and *E. raffinosus* isolates unable to spread vancomycin resistance genes from one another.
The lower intestinal tract is the most frequently colonization site especially among those with frequent hospital readmissions. They may be a well-known VRE reservoir. Skin contamination in these patients occurs readily. Numerous studies have demonstrated contamination of health care worker’s gloved and un-gloved hands. In one recent report, 29% of health care workers still had VRE on their hands after glove removal. Complicating the issue of skin colonization are the potential sequelae of enterococcal pseudo bacteraemia which result due to non-judicious use of antibiotic therapy. Therefore, the horizontal transmission of VRE in nosocomial environments can occur readily if Centres for Disease Control and Prevention guidelines to prevent the spread of vancomycin resistance genes, are not strictly followed.[25]

The glycopeptides (vancomycin and teicoplanin) are virtually the only drugs that could consistently relied on treatment of severe infections[157] caused by multidrug resistant enterococci. Vancomycin had been there in clinical use for more than 40 years; without the emergence of marked resistance. Teicoplanin is another glycopeptide antibiotic which is not available in the United States but has been used in Europe. Because of their activity against methicillin resistant staphylococci and other Gram positive bacteria, these drugs have been widely used for therapy and prophylaxis against infections due to these organisms. Oral vancomycin, which is poorly absorbed, has been use extensively for the treatment of *Clostridium difficile* enterocolitis.[27]

The first report of *E. faecalis* and *E. faecium* are resistant to high concentrations of glycopeptides antibiotics (vancomycin and teicoplanin) were published in 1988 by Uttley et al.[12] Shortly after, the first isolates of vancomycin resistant enterococci (VRE) were reported by many investigators in the United Kingdom and France.[27] There were outbreaks of vancomycin resistant *E. faecium* infections in patients of hospital renal unit located in the eastern half of the United States.[28] Subsequently, VRE have spread with unanticipated rapidity and are now encountered by many hospitals in most of the countries.[27] Glycopeptide are used for the treatment of infection with Gram positive bacteria. They form a complex with the C-terminal D-alanyl–D-alanine (D-Ala-D-Ala) of peptidoglycan precursors and block their incorporation in the cell wall thereby inhibiting cell wall synthesis.[157]
MECHANISMS OF RESISTANCE

Phenotypic Description:

There are seven recognized phenotypes of vancomycin resistance, VanA, VanB, VanC, VanD, VanE, VanG and VanD. Two of these (VanA and VanB) have mediated by newly acquired gene clusters did not previously found in enterococci. VanA and VanB resistance phenotypes described primarily in *E. faecalis* and *E. faecium*. VanA enterococci are resistant to high level vancomycin (MICs $\geq 64$ µg/ml) and teicoplanin (MICs $\geq 16$ µg/ml).\cite{27, 28}

Resistance can be induced by glycopeptides (vancomycin, teicoplanin,\cite{28} avoparcin and ristocetin) and by non-glycopeptide agents such as bacitracin, polymyxin B and robenidine (drug used to treat coccidial infections in poultry). The details of vancomycin resistance have been best documented with the *vanA* gene cluster found on the 10.8 kb transposons,\cite{158} or “jumping” genetic element, Tn1546.\cite{27,115}

VanB organisms had initially believed to be inducible resistant to more modest levels of vancomycin (MICs 32 to 64 µg/ml) but are susceptible to teicoplanin. It has now known that levels of vancomycin resistance among VanB isolates may range from 04 to $\geq 1,024$ µg/ml whereas susceptibility to teicoplanin is retained. VanB resistance determinants present on large mobile elements that can be transferred from one strain of *Enterococcus* to another.\cite{27,115}

The VanC resistance phenotype was described in *E. casseliflavus* and *E. gallinarum*, which demonstrate intrinsic, low level\cite{33,62} resistance to vancomycin (MICs 4 to 32 µg/ml),\cite{28} and are susceptible to teicoplanin.\cite{27,115} Moreover, these organisms, have low level vancomycin resistance, which is often in the “susceptible” range, as defined by criteria, which are provided by CLSI, although typically the MICs of vancomycin are higher in VanC than the MICs of non VanC enterococci.\cite{35} There are reports of failures of automated susceptibility testing systems to detect the low-level vancomycin resistance in enterococci. This may be because of its lower range (MIC 4 µg/ml) coming in susceptible criteria. Charpes PC et al.\cite{62} recommends PCR amplification of region of *vanC* gene. *E. gallinarum* may be more resistant to vancomycin than are other enterococci.\cite{100}

VanD organism are less common phenotype of acquired glycopeptide resistance,
which has been described in several isolates of *E. faecium* that were resistant to modes
Characteristics of Glycopeptide-Resistant Enterococci \[27,28,101,115,117,151\]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Phenotype</th>
<th>VanA</th>
<th>VanB</th>
<th>VanC</th>
<th>VanD*</th>
<th>VanE*</th>
<th>VanG*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin MIC (µ g/ml)</td>
<td></td>
<td>64– 1,000</td>
<td>4–1,024</td>
<td>2–32</td>
<td>64-128</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Teicoplanin MIC (µ g/ml)</td>
<td></td>
<td>16–512</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Expression</td>
<td></td>
<td>Inducible</td>
<td>Inducible</td>
<td>Constitutive</td>
<td>Inducible</td>
<td>Inducible</td>
<td>Inducible</td>
</tr>
<tr>
<td>Most frequent enterococcal Species</td>
<td></td>
<td>E. faecium, E. faecalis and A*</td>
<td>E. faecium, E. faecalis and B*</td>
<td>E. gallinarum, E. casseliflavus E. flavescens</td>
<td>E. faecium, E. faecalis</td>
<td>E. faecalis</td>
<td>E. faecium</td>
</tr>
<tr>
<td>Ligase gene</td>
<td></td>
<td>vanA cluster</td>
<td>vanB cluster</td>
<td>vanC1, C2 &amp; C3</td>
<td>vanD cluster</td>
<td>van E cluster</td>
<td>van G cluster</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td>chromosome or plasmid</td>
<td>chromosome or plasmid</td>
<td>chromosome</td>
<td>chromosome</td>
<td>chromosome</td>
<td>chromosome</td>
</tr>
<tr>
<td>PG Terminus</td>
<td></td>
<td>D-Ala- D-Lac</td>
<td>D-Ala- D-Lac</td>
<td>D-Ala- D-Ser</td>
<td>D-Ala- D-Lac</td>
<td>D-Ala- D-Ser</td>
<td>-----------</td>
</tr>
<tr>
<td>Acquired / Intrinsic</td>
<td></td>
<td>Acquired</td>
<td>Acquired</td>
<td>Intrinsic</td>
<td>Acquired</td>
<td>Acquired</td>
<td>Acquired</td>
</tr>
<tr>
<td>Transferable</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Transferee to</td>
<td></td>
<td>S. sanguis, S. pyogenes, S. aureus &amp; Listeria</td>
<td>E. faecium, E. faecalis with ampicillin resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

levels of vancomycin (MIC 64–128 µg/ml) and teicoplanin (MIC, 4 µg/ml). In addition, isolates of VanE E. faecalis and VanG E. faecium were inducible and resistant to a low concentration of vancomycin (MIC 16 mg/ml) but susceptible to teicoplanin.[28] 

There are some limitations of this classification method. For example, the genetic determinants of the VanA phenotype have now appeared in E. gallinarum and other enterococcal species. In a strain of E. avium, the VanA resistance determinants conferred a typical level of resistance to teicoplanin but low level resistance to vancomycin (MIC 16 µg/ml). Furthermore, some VanB derived mutants may exhibit resistance to teicoplanin and indistinguishable from VanA phenotype. Nevertheless, this phenotypic classification scheme is still useful, because it usually corresponds well to the genotypic classification and utilizes information that can derive simply and inexpensively in a laboratory.[27]

**GENOTYPIC CLASSIFICATION AND RESISTANCE MECHANISMS**

**Action of Vancomycin on Peptidoglycan Synthesis:**

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 then adds to UDP-N-acetylmuramyl-tripeptide to form the UDP-N-acetylmuramyl-pentapeptide. The UDP-N-acetylmuramyl-pentapeptide, when incorporated into the nascent peptidoglycan (transglycosylation), permits the formation of cross-bridges (transpeptidation) and contributes to the strength of the peptidoglycan layer. 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.[27,115]

i. **VanA glycopeptide resistance**

The vanA gene and other (vanR, vanS, vanH, vanX and vanZ) genes cluster involved in the regulation and expression of vancomycin resistance are located on 10,581-bp transposons (Tn1546) of E. faecium. Tn1546, is a 10.8 kilobase non-conjugative transposons that has been Tn5382 has been reported to be part of a larger element that carries PBP5 (mediating high level penicillin resistance), along with the vanB cluster. A number of geographically diverse VanB-type isolates had shown to co-
transfer vancomycin resistance by conjugation\textsuperscript{[154]} and resistance to high concentrations of ampicillin.\textsuperscript{[28,33]}
This cluster of genes encodes for an alternate biosynthetic pathway for the production of cell wall precursors that bind vancomycin poorly. Expression of these genes results in the synthesis of abnormal peptidoglycan precursors terminating in D-alanyl-D-lactate (D-Ala-D-Lac) instead of D-alanyl-D-alanine (D-Ala-D-Ala). Vancomycin binds to D-Ala–D-Lac with markedly lower affinity (0.001 times) than it does to the normal dipeptide product. Several proteins that sense the presence of the drug (or an effect of the drug), produce a drug resistant target, and eliminate the drug susceptible target in a coordinated manner accomplish this alteration of the target site for glycopeptides antibiotics. The core protein functions favouring synthesis of pentadepsipeptide terminating in 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. (ii) VanH protein, is a D-hydroxy acid dehydrogenase, which converts pyruvate into D-lactate for use in the above reaction. (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. (iv) VanY is an accessory structural protein that removes the terminal d-Ala residue from the PG precursor. This carboxypeptidase augments glycopeptide resistance by removing residual vancomycin binding sites. (v) The function of the VanZ protein has not understood, but it contributes to teicoplanin resistance. Like the native PG precursors, these modified precursors are polymerizing into functional cell wall.[27,28,39,40,101,115]

VanA alone cannot confer resistance to vancomycin, probably because; D-hydroxy acids such as D-Lac are neither natural product present in the environment of enterococci nor normally produced by enterococci. Thus, to synthesize D-lactate, enterococci must acquire the gene(s) within the vanA operon required to produce the substrate for VanA. VanH is responsible for the synthesis of D-lactate.[27] VanR (response regulator) and VanS (histidine kinase sensor) proteins constitute a two-component regulatory system that regulates the transcription of the vanHAX gene cluster. VanS apparently functions, act 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.
**B. Mechanism of Vancomycin Susceptible, Resistance and Dependent**

*Without Vancomycin*

**A.** Normal pathway without vancomycin. 
- D-Ala \(\rightarrow\) D-Ala
- **Cell wall**

**B.** Inhibition of cell wall synthesis
- **Vancomycin Susceptible**
- **Vancomycin**

**C.** Vancomycin Resistance
- VanA
- VanX
- VanY
- D-Ala \(\rightarrow\) D-Ala
- **Vancomycin**

**D.** Vancomycin Dependent
- VanD
- No production of D-Ala or D-Lac
- **No Vancomycin**

**Figure** - Representation of the different pathways for cell wall synthesis [peptidoglycan (PG) precursors] (A) Normal pathway without vancomycin. (B) Produces the native PG precursor that is the target for vancomycin. (C) The altered PG precursor produced by the lower pathway, binds located to plasmid and chromosomal DNA. The vanB cluster may also be present on the chromosome or plasmid DNA and thus far has been found in 2 different transposons, Tn1547 and Tn5382. vancomycin poorly present in a VanA Enterococci. (D) Loss of Production of either D-Ala or D-Lac, in Vancomycin dependent enterococci. VanX, VanY and VanH encoded by the vanA gene cluster, modifies the finished native PG precursor.\(^{[28,54,114]}\)
In VanA phenotype strains, either vancomycin or teicoplanin can induce the transcription, but the precise signals are still unknown. vanY and vanZ may contribute to (but not essential) resistance. VanY protein is a D,D-carboxypeptidase that cleaves the D-Ala terminal peptide from any normal peptide that may have been made contributing modestly to resistance levels. VanZ modestly increases the MICs of teicoplanin but not of vancomycin, through mechanisms that have not yet been elucidated. It is not essential for expression of the VanA phenotype. Tn1546 existed intact in some strains but had insertion like sequences between vanS and vanH in others. These vancomycin resistance gene clusters may be incorporated into even larger mobile elements containing additional insertion-like elements\textsuperscript{[27,28]} Primers specific for regions of Tn1546 flanking vanRS, vanSH, vanHAX, vanXY and vanYZ were used to amplify these areas and are shown in following table.\textsuperscript{[158]}

Sequences of primers used to amplify vanA genes in PCR experiments\textsuperscript{[158]}

<table>
<thead>
<tr>
<th>Primer</th>
<th>Amplicon</th>
<th>Primer sequence (5’€3’)</th>
<th>Amplicon size of EF228 (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vanR1</td>
<td>vanS2</td>
<td>ATGAGCGATAAAAAATACITT TTAGGACCTCCTTTTATC</td>
<td>1.8</td>
</tr>
<tr>
<td>vanS1</td>
<td>vanH2</td>
<td>TTAGGTTATAAAAATTGAAAATT CTATTCACTGCTCTGCTCT</td>
<td>2.3</td>
</tr>
<tr>
<td>vanH1</td>
<td>vanX2</td>
<td>ATGAATAACATCGGCATTAC TTATTTAACCGGGGAAATC</td>
<td>2.6</td>
</tr>
<tr>
<td>vanX1</td>
<td>vanY2</td>
<td>ATGGAAATAGGTATTACTTT TTACCTCCTTGAATTAGTAT</td>
<td>1.9</td>
</tr>
<tr>
<td>vanY1</td>
<td>vanYZ</td>
<td>ATGAGAAGTTGTTTTTTTTTA vanZ2 CTTACACGTAATTATTAC</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Cross-linking of the precursors to the growing peptidoglycan is processed in bacteria by the PBPs, with PBP5 being used in enterococci. The replacement of D-Ala by D-Lac does not impair cross-linking of the modified precursors to the growing peptidoglycan chain. However, PBPs other than PBP5, which are not known to play a role in cell wall synthesis, are probably required for processing of the altered precursors. These high-molecular-weight PBPs display a higher affinity for $\beta$-lactams. Since VanA resistance is inducible, a shift in the PBPs occurs only in the presence of vancomycin and results in $\beta$-lactam hyper susceptibility. This effect explains the synergy displayed by the combination of the two classes of drugs against vancomycin-resistant strains.\textsuperscript{[27]}
Enterococcal VanB glycopeptide resistance is mediated by an abnormal ligase (VanB) that is structurally related to VanA ligase (76% amino acid identity). VanB protein also favours the production of the pentadepsipeptide terminating in D-Ala–D-Lac. Genes analogous to their VanA resistance counterparts are designated vanHB, vanXB, vanYB, vanRB and vanSB. Levels of D,D-dipeptidase activity (VanXB) correlate with levels of vancomycin resistance.\textsuperscript{[27,101]} There is a high degree of sequence identity (approximately 70%) between VanHAX and VanHBXB but considerably less homology (25 to 35% sequence homology) between the RS and Y proteins of VanA and VanB VRE. There is no gene counterpart of vanZ in these organisms.\textsuperscript{[115]} vanYB is not found in all strains, and its position in the gene clusters differs from that of vanY in Tn1546. vanW is present but function of it is still unknown.\textsuperscript{[28]}

Recent reports have shown DNA sequence heterogeneity, suggesting three subtypes of the vanB ligase gene: vanB-1, vanB-2, and vanB-3. The regulatory system in class B strains appears insensitive to induction by teicoplanin. Teicoplanin induces the synthesis of VanA related proteins but does not induce the production of VanB related proteins. Vancomycin induces the synthesis of the resistance proteins of both systems, and in fact, if a teicoplanin-susceptible enterococci with the vanB gene cluster were pre-exposed to vancomycin and then tested for teicoplanin shows resistant as well. In addition, teicoplanin-resistant mutants can be derived from teicoplanin susceptible, vanB containing enterococci when these organisms are plated onto teicoplanin containing agar. Such mutants can also arise in vivo during therapy. Possible mechanisms for teicoplanin resistance of these mutants include the loss of their requirement for an inducer (that is, if they constitutively produce high levels of the vancomycin resistance proteins) and the ability of teicoplanin to act as an inducer.\textsuperscript{[27]}

Another difference between VanA- and VanB-type resistances is that VanA is more widely distributed. It is the predominant type of resistance reported in Europe. While VanB strains are common in the United States, with some hospitals reporting VanB exclusively, VanA still predominates. The vanA ligase gene is also found in a wider range of enterococcal species as well as in Corynebacterium species, Arcanobacterium haemolyticum, & Lactococcus species, while vanB has found primarily in E. faecium & E. faecalis only.\textsuperscript{[27,115]}
The difference in the dissemination of these resistance traits may be related to the observation that the vanA gene cluster is often located on a transposon similar to Tn1546, which is the part of a conjugative (transferable) plasmid. Such a genetic arrangement is an excellent avenue for the dissemination of these genes. The vanB cluster is often located on the host chromosome and initially thought not to be transferable to other bacteria. However, it can occur on plasmids also and even when it is chromosomal, this gene cluster has been transferable as part of large mobile elements, perhaps related to large conjugative transposons.²⁷¹¹⁵

iii. **VanC glycopeptide resistance**

Low level resistance to vancomycin is typical of *E. gallinarum*, *E. casseliflavus* and *E. flavescens*. The nucleotide sequences of the vanC-1 gene in *E. gallinarum*, the vanC-2 gene in *E. casseliflavus* and the vanC-3 gene in *E. flavescens* have reported, although there is some disagreement about whether *E. flavescens* is a legitimate enterococcal species. VanC ligase of *E. gallinarum* favours the production of a pentapeptide terminating in D-Ala–D-Ser. Substitution of D-Ser for D-Ala has presumed to *weaken* the binding of vancomycin to the novel pentapeptide.²⁸¹¹⁵ Insertional inactivation of vanC-1 unmasks the concomitant production of the D-Ala–D-Ala pentapeptide in *E. gallinarum*.

D,D-Dipeptidase and D,D-carboxypeptidase activities analogous to those of VanA and VanB strains have been described. It is presumed that the level of resistance expressed represents the balance achieved between normal and abnormal peptidoglycan synthesis. The presence of variable amounts of D-Ala–D-Ala relative to D-Ala–D-Ser could account for the variable levels of vancomycin resistance observed among isolates of VRE carrying the VanC phenotype. That is, lower MICs could be explained by the presence of larger amounts of D-Ala–D-Ala, which enables vancomycin to inhibit cell wall synthesis, and higher MICs could explained by a higher proportion of D-Ala–D-Ser. This resistance may be inducible or constitutive.²⁷

The vanC-2 gene of *E. casseliflavus* demonstrates approximately 66% nucleotide sequence similarity to vanC-1. Like *E. gallinarum*, these strains also possess an additional native ligase. There is extensive homology (98%) between the gene
sequences of vanC2 and vanC3. The vanA genes have recently been identified in strains of *E. gallinarum* and *E. casseliflavus*, conferring higher levels of resistance to
vancomycin (MIC, >256 µg/ml) in these species than normally anticipated and resulting in resistance to teicoplanin.[27]

iv. **VanD glycopeptide resistance**

A novel vancomycin resistance designated gene vanD was first described in a New York Hospital in 1991.[115] The strain carrying this resistance trait was an *E. faecium* strain that was inhibited by vancomycin at 64 µg/ml and teicoplanin at 4 µg/ml.[28] Partial sequencing of the ligase gene showed that it was distinct from but similar to the vanA and vanB ligase genes. Recently, three clinical isolates of vancomycin resistant *E. faecium* carrying the VanD resistance trait, had found in Boston, and the deduced amino acid sequence of VanD showed 67% identity to those of VanA and VanB. VanD appears to be located on the chromosome and is not transferable to other enterococci.[27,115]

VanD enterococci have a mechanism of glycopeptide resistance similar to VanA and VanB microorganisms that is, the formation of D-Ala–D-Lac–terminated PG precursors mediated by a cluster of genes with homology to vanRS, vanY and vanHAX.[28]

v. **VanE glycopeptide resistance**

The vanE vancomycin resistance gene has recently been described in *E. faecalis* (BM4405) and is due to synthesis of late peptidoglycan precursor ending in D-Ala-D-Ser. VanE is resistant to low levels of vancomycin (MIC 16 µ g/ml) and susceptible to teicoplanin (MIC 0.5 µ g/ml). This new resistance phenotype has similarities to the intrinsic VanC type of resistance. The deduced amino acid sequence has a greater identity to VanC[115] (55%) than to VanA (45%), VanB (43%), or VanD (44%).[24] The vanE gene cluster in BM4405 is still under study.[159]

vi. **VanG glycopeptide resistance:**

VanG vancomycin resistance gene is recently found in *E. faecium*. It is coded on chromosome.[27]

vii. **Other resistance classes and organisms**

*Lactobacillus casei, Pediococcus pentosaceus* and *Leuconostoc mesenteroides*
are naturally resistant to glycopeptides. Although the terminus appears to be the same
(D-Ala–D-Lac), as found in VRE with VanA or VanB phenotypes, DNA from these organisms does not hybridize with resistance gene probes prepared from VRE.\textsuperscript{[27]}

In the laboratory, conjugal transfer of VanA type vancomycin resistance genes from enterococci to other Gram positive cocci has been accomplished. Recipient organisms in successful transfers have included group A and viridans group streptococci, \textit{Listeria monocytogenes} and \textit{Staphylococcus aureus}.\textsuperscript{[19]} Transfer of resistance genes to \textit{S. aureus}, resulting in high levels of resistance to vancomycin, was demonstrated both in vitro as well as in vivo (on the skin of mice). This gives rise to concern that such transfer in humans under natural conditions indeed might be feasible. Vancomycin resistance genes have already found in human isolates of non-enterococcal organisms. A \textit{vanB}-related gene sequence (designated \textit{vanB3}) has found in \textit{Streptococcus bovis}.\textsuperscript{[27]}

\textit{Vancomycin-dependent enterococci (VDE):}

Vancomycin is poorly absorbed from the gastrointestinal tract after oral administration, and concentrations can exceed 30 mg/g of faeces at doses of 2 g daily. It is not surprising that this environment promotes the selection of organisms resistant to vancomycin.\textsuperscript{[54]} An interesting phenomenon that has developed in some strains of VanA- and VanB-type VRE by mutation leading to vancomycin dependence. These enterococci are not just resistant to vancomycin but they require it for their growth.\textsuperscript{[115]}

Vancomycin dependent enterococci have been recovered from apparently culture negative clinical samples by plating them onto vancomycin containing agar (used for isolation of \textit{Campylobacter} or gonococci).\textsuperscript{[27,28,54]} Prior exposure, often protracted, to vancomycin and other broad spectrum antibiotics is a frequent finding in the VDE related literature. Patients exposed to “antibiotic pressure” with vancomycin and probably extended spectrum cephalosporins and antianaerobic agents are at increased risk for colonization as well as infection with VRE. The antibiotic pressure plays an impotant role in promoting colonization or infection with VDE.\textsuperscript{[50]}

The Lisa Dever\textsuperscript{[160]} reported that initial VREF stool isolate from patient had the same biochemical reactions and antibiotic susceptibility patterns as VDEF and it is reasonable to suspect that the VDEF isolate arose from it in the presence of

\textbf{~ 126 ~}
vancomycin. Some time isolation of Enterococci is dependent on vancomycin for their growth from the urine or bloods of patients those receiving intravenous vancomycin. Vancomycin-dependent isolates in these reports were of the vanB genotype. Rosato and co-workers also recently described the selection of a vancomycin dependent mutant from a clinical isolate of a VanA type Enterococcus avium.\textsuperscript{[54]} Bambeke FV reported not only vancomycin but also teicoplanin dependent mutant by growing them in the presence of vancomycin 10 µg/ml and teicoplanin 1 µg/ml. All these mutants showed the same resistance phenotype to antibiotics except glycopeptides as shown by the parental strains.\textsuperscript{[161]}

In each of these cases, investigators proposed that vancomycin dependent enterococci might lack a functional D-alanyl–D-alanine ligase. DNA sequencing has detected DDL gene mutation (e.g. P175L) or deletion (e.g. 1 bp deletion of D-alanyl–D-alanine ligase gene or 18 bp deletion of VanSB gene) among VDE strain.\textsuperscript{[50,162]} Molecular mechanisms for the development of VDE have been recently described. Vancomycin inhibits the growth of vancomycin-susceptible enterococci by binding to the normal bacterial cell wall dipeptide D-Alanyl D-Alanine (“D-Ala D-Ala”) peptidoglycan. 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 making. 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 \textbf{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 \textbf{destroyed} by the action of \textbf{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. Because of amino acid substitutions or deletions in “D-Ala–D-Ala” ligases, however, VDE are unable to make the original “d-ala d-ala” cell wall dipeptide. Its growth is thus dependent on the presence of vancomycin, which enables the bacteria to use “D-Ala D-lac” as a cell wall constituent.\textsuperscript{[27, 28,50,54,115]}
Photograph. F

Growth of vancomycin dependence of *E. faecium* isolate.

(i) Growth around a 30-µg vancomycin disc on Mueller-Hinton agar supplemented with blood. A large spontaneous reverting colony not requiring vancomycin for growth is showing adjacent to the cephalothin (CF) disc. There is no growth around teicoplanin disc. \[^{[54,160]}\]

(ii) Blood agar shows growth around a disk containing 1,000 µg of D-alanyl–D-alanine. Growth was less optimal with D-alanyl–L-tha vancomycin. Large spontaneous revertant colonies not requiring D-alanyl–D-alanine for growth can be seen away from the disc. \[^{[54,160]}\]

(iii) VDE on left side, and Control on right (and Control) is vancomycin sensitive. \[^{[162]}\]
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.[27,28,54,115] Reversion of VDE to vancomycin independence occurs at varying rates, from 1:10^2 colonies (vanA isolates) to 1:10^7 colonies (vanB isolates), which results in a phenotypically mixed population.[50]

Growth may be supported by compounds that either induced the production of the vanB ligase or can be substituted for the products of the missing native ligase. For example, ristocetin, a glycopeptide closely related structurally to vancomycin, most likely induces the production of the vanB ligase. In contrast, the dipeptide D-alanyl-D-alanine is likely to incorporated into peptidoglycan precursors substituting for the D-alanyl-D-lactate made by the vanB ligase.[54] The growth of these VDE organisms can be supported by vancomycin (0.125 to 50,000 µg/ml), ristocetin and D-alanyl-D-alanine, (shown in photograph) but not supported by the glycopeptide antibiotic teicoplanin, daptomycin or D, L-alanine.[160,162] The spontaneous revertants away from vancomycin dependence can occur at frequency of 1 in 10^6.[161] Awareness of the existence of these strains is important, especially in the context of long-term vancomycin therapy and suspected presence of nutritionally deficient organisms.[162]

The exact prevalence of vancomycin dependent enterococci is not known. In fact, the recent flurry of reports suggests that clinical isolates of vancomycin-dependent enterococci may increasingly recognized when appropriate culture techniques are used. Although routine screening for vancomycin-dependent enterococci in patients receiving vancomycin has not indicated, it may warrant for culture negative patients who have continuing evidence of infection and have previously had positive cultures for vancomycin resistant enterococci. In these situations, additional specimens should be obtained and cultured on either media containing vancomycin or media with vancomycin disks. Lisa L Dever[160] described a faecal VREF that became dependent on vancomycin for growth during oral therapy. The versatility of the enterococci under the extremes of antibiotic pressure and provide support for recommendations to limit the use of both oral and intravenous vancomycin.[54]

Emergence of vancomycin dependent strains in clinical settings currently appears to be more of a curiosity than problem of clinical concern. The inability of these bacteria to survive in the absence of vancomycin makes them a priority, easy to
eliminate, simply by discontinuing antibiotic therapy. However, vancomycin dependent enterococci are rarely isolated in routine laboratory practice because of their particular nutritional requirements. Growth is obtained only on medium if it is supplemented by either D-Ala-D-Ala, vancomycin or other inducers of vancomycin resistance gene expression (e.g. bacitracin and monomycin). Moreover, Vancomycin dependent mutants have also been isolated from patients not receiving vancomycin. Therefore, they constitute a reservoir of vancomycin resistance genes which could be transferred to other bacteria. More importantly, Vancomycin dependent strains can easily revert, at least in vitro, to a nondependent, highly resistant phenotype and may therefore require particular attention. Screening for Vancomycin dependent isolates in infected patients is therefore advisable.\textsuperscript{161}

**Source of Vancomycin Resistance Gene:**

The vancomycin resistance present in non-enterococcal organisms may have been transferred to enterococci under the pressure of increased oral and parenteral vancomycin use in clinical practice and use of glycopeptides (avoparcin and / or ristocetin) in animal husbandry. The exact source of vancomycin resistance genes is unknown. It is recently hypothesized that the source may be glycopeptides producing organism. Other environmental organism may have been the more direct source.\textsuperscript{163}

The \textit{vanHAX} genes have an arrangement identical and significantly predicted amino acid–sequence similarity to those of genes found in \textit{Streptomyces toyocaensis} and \textit{Amycolotopsis orientalis}, actinomycetes that produce glycopeptide antibiotics. Substantial differences between the guanine-cytosine (G-C) content of these two groups of homologous genes suggest that a recent transfer of vancomycin resistance genes from these antibiotic producers to enterococci has not occurred. Another, more closely related group of \textit{vanHAX} homologues (based on deduced amino acid–sequence homology and G-C content) is found in the vancomycin resistant biopesticide \textit{Paenibacillus popilliae}. The vancomycin resistance gene cluster in \textit{Paenibacillus popilliae} is more similar to that in VRE than are gene cluster in \textit{Streptomyces toyocaensis} and \textit{Amycolotopsis orientalis}. The gene cluster present in \textit{Paenibacillus popilliae} has homology to VanA (VanB and VanD) gene clusters of enterococci. The partial sequence of VanE in \textit{E. faecalis} BM4405 has predicted amino acid identity to VanF in \textit{P. popilliae}.\textsuperscript{28,163}
Paenibacillus popilliae spores have been introduced into soil in the eastern United States as a biopesticidal powder since the early 1940s. It is available in market as Milky Spore (does not affect humans, animals or contaminated well water). Once established in a lawn, they survive for 15 to 20 years. It is suggested that spread of Paenibacillus popilliae spores may have accomplished by birds, insects, skunks, moles and mice. Such wide spread distribution of this organisms may have been provided opportunity for its contact with enterococci. Furthermore, both enterococci and Paenibacillus popilliae are able to survive for long period in the environment even though some adverse conditions.[163]

The several intrinsically vancomycin resistant, Gram positive organisms, including Pediococcus species, Leuconostoc species and some lactobacilli also produce PG precursors that terminate in D-Lac. However, the D-Ala:D-Lac ligases found in these organisms are only distantly related to the VanA, VanB, and VanD ligases. Because of differences in G-C content, it has been hypothesized that the regulatory genes (vanR and vanS) and the accessory genes (vanY and vanZ) may be derived from a different source than the essential structural genes (vanHAX). The regulatory genes of the vanA and vanB clusters show substantially lesser homology to each other than vanHAX does to vanHB BXB.[28]

**Mobility of Element Containing Genes for Vancomycin Resistance**

The situation is complicated by the fact that enterococci have developed a number of mechanisms for the transfer of resistance genes. Therefore, perhaps the furthermost threat posed by VRE is not from enterococci but from the potential that they could transfer their resistance genes to other more pathogenic Gram positive bacteria, thus creating a highly dangerous pathogen difficult to treat with currently available antibiotics.[164]

Enterococci have various systems of bacterial mating (conjugation) that can spread the resistance gene to other bacteria. These systems include plasmid that can replicate in several other Gram positive species (e.g. Staphylococci and Streptococci). Pheromone responsive plasmid that can be transferred between E. faecalis strains at frequencies some time approaching 100%, and specialized type of transposons (an element that can jump from one DNA site to another intra-cellular), or conjugative (that
is, it can transfer inter-cellular between broad range of bacterial genera and can then integrated into the genome of the new host bacterium).\cite{117}

The finding of genes for vancomycin resistance on these conjugative as well as transferable elements heightens concern about possible transfer of such resistance to other, perhaps more pathogenic, organisms. Such concern reports of the experimental or may be natural transfer of VanA vancomycin resistance from enterococci to \textit{Staphylococcus aureus}, \textit{Listeria monocytogenes},\cite{114} \textit{Streptococcus pyogenes}\cite{117} and \textit{Streptococcus sanguis}\cite{115} \textit{Lactococcus lactis}. In addition, laboratory experiments, the VanA gene has been found in vancomycin resistant clinical isolates \textit{Cellulomonas turbata}, \textit{Acanobacterium haemolyticum} and \textit{Bacillus circulans}, while VanB is found in vancomycin resistant isolates of \textit{Streptococcus bovis}.\cite{163} The experimental transfer of vancomycin and ampicillin resistance together by conjugation between strains \textit{E. faecium} has reported. Nevertheless, such transfer to \textit{E. faecalis}, streptococci or pneumococci would have serious consequences clinically.\cite{117} This gives rise to concern as such transfer in human and in environment under natural condition might be feasible.

**Status of VRE in India**

The problem of VRE is not very high in India, but monitoring of VRE is needed, since it appears to be an emerging pathogen in India.\cite{30} In a study conducted at All India Institute of Medical Sciences, New Delhi, by the disk diffusion and agar screen methods five isolates of \textit{E. faecalis} were found to be resistant to vancomycin. On PCR, four had VanA phenotype and one had VanB phenotype. In another study from Lady Hardinge Medical College, New Delhi, Chandigarh and Mumbai reported 8, 5.5 and 23 percent VRE respectively, and all being Van B phenotype.\cite{115}

**Antibiotic Synergism**

Antibiotic resistance among enterococci is a major obstacle to therapy. Many studies have reported that enterococci are intrinsically resistant to penicillins, cephalosporins, clindamycin and aminoglycosides. In contrast to uncomplicated enterococcal infections such as urinary tract and wound infections, which respond to ampicillin but serious enterococcal infections such as endocarditis, meningitis require
combinations of these antibiotics which synergistically kill enterococci $^{[29]}$ and they are recommended as the treatment of choice.$^{[132]}$
Standiford HD (1970) identified that enterococci became resistant to streptomycin or kanamycin and MICs were >2,000 µg/ml (high level aminoglycoside resistance). Penicillin in combination with the aminoglycoside has not synergistic killing effect against high level resistant enterococci.[29]

Enterococci are relatively impermeable to aminoglycoside antibiotics, and that the permeability barrier can be breached by other agents, which inhibit bacterial cell wall synthesis. The uptake of this aminoglycoside antibiotic was markedly enhanced in enterococci growing in the presence of penicillin or other agents, which inhibit the synthesis of bacterial cell walls. There was no enhancement of streptomycin uptake when the cells were incubated with antibiotics, which primarily affect the bacterial cell membrane or inhibit protein synthesis. Uptake of streptomycin is increased by penicillin, only in actively growing bacteria. These observations are consistent with the hypothesis that enterococci exhibit a natural barrier to the entry of streptomycin, which can overcome by agents, which inhibit cell wall synthesis, thus producing a synergistic effect. To test the validity of this hypothesis, Robert CMet al.[165] have studied the uptake of "C-labelled streptomycin by enterococci in the presence of various antibiotics.

The rationale for this approach depends on (i) the ability of the cell wall active antibiotic to modify the architecture of the cell wall and increase permeability of the aminoglycoside antibiotic, (ii) the susceptibility of the ribosome to attachment and inhibition by the aminoglycoside, and (iii) the presence of aminoglycoside inactivating enzymes. If cell wall integrity is destroyed by a cell wall active agent, the lesser concentrations of aminoglycoside should effectively bind 30 S portions of the affected ribosome.[134] However emergence of high level aminoglycoside resistance (HLAR), β-lactam antibiotics & vancomycin by some strains, together with association of HLAR with multi drug resistance has led to failure of synergistic effect of combination therapy.[30]

Combination of penicillin and streptomycin produce bactericidal killing of enterococci until unfortunately, they develop high level streptomycin resistance, which is either ribosomal mediated or due to the production of aminoglycoside inactivating enzymes. It is very important to test susceptibilities to both agents because enterococcal resistance to gentamicin and streptomycin occur by different mechanisms. Gentamicin resistance is predominantly the result of the presence of the inactivating enzyme 2"
phosphotransferase and 6'acetyltransferase conferring resistance to gentamicin, tobramycin, netilmicin, amikacin and kanamycin. Hence, gentamicin resistance is a good indicator of resistance to other aminoglycosides except streptomycin. Streptomycin resistance is encountered mainly in enterococcal strains that produce streptomycin 6' adenyltransferase; these strains remain susceptible to gentamicin. Some isolates produce 3'' phosphotransferase which inactivates kanamycin and amikacin become resistant to these antibiotics, but may be susceptible to gentamicin and other aminoglycosides. Penicillin-aminoglycoside synergy does not occur in high level aminoglycoside resistant enterococci (streptomycin MIC ≥2,000 mg/ml; gentamicin MIC ≥500 mg/ml).[27,32, 46,115]

Resistance to a number of antimicrobial drugs is a characteristic of the genus Enterococcus, although some species (e.g. E. faecium) are more intrinsically resistant than others. E. faecium carries aac(6')-Ii, a chromosomal gene encoding an aminoglycoside modifying enzyme that prevents synergy between cell wall active agents and the aminoglycosides tobramycin, kanamycin and netilmicin.[28]

Through mutation and transfer of resistance genes (transferable plasmid) from other species (and in some cases between enterococcal species), enterococci have acquired additional resistance determinants. The transferable high level gentamicin resistance in Enterococcus faecium predicted the spread of this resistance to other isolates and species of enterococci. Subsequent reports have confirmed the dissemination of high level aminoglycoside resistance, including resistance to gentamicin among E. faecium and other enterococcal species.[39] E. faecium often (>60%) displays acquired resistance to penicillin that are substantially higher than the microorganism is intrinsically able to resist, mediated by increased expression of low-affinity penicillin binding protein 5 (PBP5) or mutations in PBP5 that produce progressively lower affinity for penicillin in the most resistant strains. β-Lactamase is essentially identical to the one that has found in Staphylococcus aureus causes and E. faecalis.[28]

Such enterococci were refractory to combination therapy in the rabbit endocarditis model and were responsible for clinical failures in human endocarditis when treatment with penicillin and the relevant aminoglycoside was employed. The percentage of enterococci showing high level resistance to streptomycin and kanamycin.
has increased in major United States cities, up to 25 to 55% of isolates are streptomycin resistant and 15 to 50% are kanamycin resistant.\textsuperscript{[28,29,132]}

Horodniceanu \textit{et al.} (in 1979) reported the first \textit{Enterococcus faecalis} isolates highly resistant to gentamicin [gentamicin-resistant enterococci (GRE)] from France.\textsuperscript{[29,30]} Published reports in 1983 from Houston, Bangkok and Chile and in 1986 from Michigan, documented a GRE prevalence of 4.5, 14, 15 and 13 and 55%, respectively, among recent clinical isolates. Ten GRE isolates (reported from three centres) were susceptible to streptomycin but highly resistant to all other aminoglycosides tested.\textsuperscript{[29]}

Enterococci have become increasingly important not only because of their increase resistance to many antimicrobial agents but also serious enterococcal infections are often refractory to treatment and the morality is very high. The probability that synergy resistant enterococci will emerge in many health care facilities, coupled with the serious consequences, this resistance might create complications in the therapeutic management of patients.\textsuperscript{[132]} The infections caused by enterococci have traditionally been treated with cell wall active agents (e.g. penicillin or ampicillin) in combination with an aminoglycoside\textsuperscript{[30,134]} (streptomycin / gentamicin), which results in higher cure rates than when one agent is used alone.\textsuperscript{[134]}

High level resistance is reported for every aminoglycoside that might be considered for combination therapy; these include streptomycin, kanamycin, amikacin, tobramycin, gentamicin, and netilmicin. The emergence of these resistant strains can significantly limit the therapeutic choices for serious enterococcal infections.\textsuperscript{[132]}

The consequence that such resistance may have effect on synergistic therapy of serious enterococcal infections and emphasizes the need to establish in vitro method for accurate resistance detection\textsuperscript{[39]} for enterococci and those which predict the susceptibility of the organism to synergy.\textsuperscript{[132]}
PREVENTION AND CONTROL

Recently, enterococci have become more resistant to antimicrobial agents; therapeutic options are becoming increasingly limited. Multiple drug resistant enterococci have become an increasing threat, with some isolates resistant to all possible microbial choices. As resistance increases, the control of emergence and spread of organisms become more imperative. Although most outbreaks have been controlled with strict application of barrier precautions, a multipronged approach may be necessary in many cases. To decrease risk of colonization of enterococci we have to take the essential steps in limiting the spread of both sensitive and resistance enterococci by limiting the injudicious use of antimicrobials.[22]

Vancomycin Resistant Enterococci (VRE)

Vancomycin resistant enterococci have alarmed the global infectious diseases community for several reasons. First, enterococcal acquirement of vancomycin resistance leaves very few options for treatment. Secondly, conjugation experiments have confirmed that vancomycin resistance gene transfer from enterococci to Staphylococcus aureus and other organisms. Third, epidemiological studies in the United States and Europe have recognized dissimilar selection pressures for VRE explosion, yet there is analogous and speedy spreading out of resistant populations. Finally, the limited successes over the past three decades of prevention and control strategies for containing vancomycin resistance highlight the difficulty of limiting the problem once it is established.[25]

VRE are opportunistic pathogens, considerable multiple drug resistant as well as survive in the hospital environment. Enterococci are versatile organisms that survive on the hands of health care workers and on inanimate objects. VRE have been demonstrated in the stool of colonized patients, sometimes for extended periods.[28]

There are recognized tensions and controversies surrounding the current
recommendations to prevent and control the spread of vancomycin resistance enterococci. There are gaps in our existing knowledge of enterococcal virulence especially *E. faecium* and *S. aureus*. This threat is further substantiated by the recognition of identical transposons in enterococci and *S. aureus* and our limited ability
in nosocomial settings to control the spread of either VRE or MRSA. In preparation for the emergence of vancomycin resistant *S. aureus*, the Centres for Disease Control and Prevention have outlined an extensive plan that could readily be adopted or modified by health care institutions across the country.\[27\]

There are such resource intensive efforts which are realistic, given the limited research and financial allocations directed towards this goal. Numerous basic science, applied research and epidemiological studies have concluded and provided an outline for the Hospital Infection Control Practice Advisory Committee recommendations for prevention of the spread of vancomycin resistance genes. These recommendations include i. surveillance, ii. applied research, iii. prevention and control measures and iv. development or expansion of infrastructure.\[25\] Each component in detail is given below.

i. **Surveillance:**

Surveillance strategies are two types like active surveillance and passive surveillance, these are depending upon the function and accessible resources. Active surveillance includes the probable specimen collections for baseline studies and follow-up evaluation of disease burden. While, passive surveillance occurs in most health care settings when specimens routinely collected for clinical care can be further assessed for infection control purposes. A few hospitals regularly screen enteric *Clostridium difficile* specimens for VRE surveillance. In a recent study, evaluating VRE detection in stool specimens submitted for toxin production in *C. difficile*, there was a 19% detection rate for VRE compared to 13% for *C. difficile* toxin.\[25,154\]

ii. **Applied Research:**

Molecular methods can provide supportive evidence for epidemiological findings. Methods like PCR and pulsed-field gel electrophoresis can be used for identification of a new strain(s), dissemination of vancomycin resistant genetic elements, outbreaks due to the spread of a single clone, and confirmation of initial clonal spread followed by establishment and maintenance of an endemic state caused by enterococci. Coordinated investigations of clinical outcomes, linked with enterococcal virulence assessment, would further clarify the current enigmas regarding the significant risks for death associated with enterococcal bacteraemia.\[25,27\]
iii. Prevention and Control:
Appropriate infection control strategies are required to prevent the spread of multidrug-resistant organisms. Medical centres detecting their first cases of VRE should be particularly aggressive in implementing infection control to prevent the organisms from becoming endemic. Once VRE have establish as endemic area, then infection control becomes more and more complicated.\[28\]

The resource utilizations are more appropriate method for control of the infection. In addition, these recommendations for acute care are more rigorous than, those for long-term care. Regardless of the clinical setting, prevention and control strategies is the practice of hand washing. Numerous studies have evaluated health care worker behaviours and noted major and minor violations in hand washing techniques.\[27\]

Perhaps most important in the prevention and control of the spread of VRE is the recognition of the role that faecal carriage has in colonization pressure. Among the known independent risk factors for VRE acquisition are extended length of stay, higher severity of illness scores, colonization pressure, and prolonged antimicrobial exposure, and thus measures to reduce or enhance these risks should be incorporated into routine clinical care.\[27\]

iv. Development or Expansion of Infrastructure:

Over the past three decades, there has been growing recognition of the economic impact of nosocomial infections across the continuum of care, which compounds the substantial costs of health care in general. Despite the ongoing development and expansion of technological advances within health care delivery systems, pressures to reduce costs focus on downsizing programmes, reducing waste, and limiting resource utilization. Ideally, a core infrastructure is needed in infection control programs that have linked to microbiology, pharmacy, and an informatics system.\[25\]

**INTERRUPTION OF TRANSMISSION**

The epidemiology of VRE has not elucidated completely. However, certain patient populations are at increased risk for VRE colonization or infection. The enterococci are part of the normal flora and most infections with these organisms have
attributed to the patient’s endogenous flora. However, recent reports have demonstrated that enterococci, including VRE, can spread by direct patient-to-patient contact or
indirectly via transient carriage on the hands of personnel, contaminated environmental surfaces or patient care equipment.\textsuperscript{[27]}

In the context of dramatic increase in vancomycin resistance in enterococci, the Subcommittee on Prevention and Control of Antimicrobial Resistant Microorganisms in Hospitals of the CDC’s Hospital Infection Control Practices Advisory Committee (HICPAC) had several meetings in 1993 and 1994. HICPAC published their recommendations to control the nosocomial transmission of VRE (February 1995).\textsuperscript{[27]}

These recommendations mainly focused on

- ii. Prudent use of vancomycin,
- iii. Education of hospital staff,
- iv. Effective use of the microbiology laboratory and
- v. Implementation of infection control measures. Aim of this recommendation is to minimize nosocomial transmission of VRE. Hospitals must use a multidisciplinary approach that requires participation by various departments and personnel.

\textbf{i. Prudent use of Vancomycin}

Appropriate use of vancomycin and 3\textsuperscript{rd} generation cephalosporin is necessary to decrease the spread of organisms between individuals and hospital. The logic behind efforts is to decrease use of antibiotic. Because, the presence of an antibiotic provides a tremendous advantage to a resistant organism and can increase the number of resistant bacteria many fold. The number of resistant bacteria present in clinical sample, the easier to transmit them to another person.\textsuperscript{[27]}

Encouraging the appropriate use of oral and parenteral vancomycin is an important component of HICPAC recommendations.\textsuperscript{[115]} In an effort to bring about more prudent use of antibiotics, HICPAC emphasizes the importance of education of medical staff and students about appropriate use of vancomycin.\textsuperscript{[27]}

\textbf{Situations in which the use of vancomycin is appropriate or acceptable:}

- i. For treatment of serious infections due to $\beta$-lactam resistant Gram positive microorganisms.
- ii. For treatment of infections due to Gram positive microorganisms in patients with serious allergy to $\beta$-lactam antimicrobials.
- iii. When antibiotic-associated colitis fails to respond to metronidazole therapy or is
severe and potentially life threatening.
iv. Prophylaxis, as recommended by the American Heart Association, for endocarditis following certain procedures in patients at high risk for endocarditis.

v. Prophylaxis of major surgical procedures, involving the implantation of prosthetic materials or devices, e.g. cardiac and vascular procedures and total hip replacement, at institutions with a high rate of infections due to methicillin resistant *S. aureus* (MRSA) or methicillin resistant *S. epidermidis* (MRSE). A single dose administered immediately before surgery is sufficient unless the procedure lasts more than 6 hour, in which case the dose should repeated. Prophylaxis should discontinue after a maximum of two doses.

**Situations in which the use of vancomycin should be discouraged:**

i. Routine surgical prophylaxis other than in a patient with life threatening allergy to β-lactam antibiotics.

ii. Empirical antimicrobial therapy for a febrile neutropenic patient, unless there is strong evidence at the outset that the patient has an infection due to Gram positive microorganisms (e.g. inflamed exit site of a Hickman catheter) and the prevalence of infections due to MRSA in the hospital is substantial.

iii. Treatment in response to a single blood culture positive for coagulase-negative staphylococci, if other blood cultures drawn in the same period are negative i.e., if contamination of the blood culture is likely (because contamination of blood cultures with members of the skin flora, e.g. *S. epidermidis*, may cause vancomycin to be administered to patients inappropriately, phlebotomists and other personnel who obtain blood cultures should be trained properly to minimize microbial contamination of specimens).

iv. Continued empirical use for presumed infections in patients whose cultures are negative for β-lactam-resistant Gram-positive microorganisms.

v. Systemic oral or local (e.g. antibiotic lock) prophylaxis of infection or colonization of indwelling central or peripheral vascular catheters.

vi. Selective decontamination of the digestive tract.

vii. Eradication of MRSA colonization.

viii. Primary treatment of antibiotic associated colitis.

ix. Routine prophylaxis in very low birth weight infants.

x. Routine prophylaxis in patients on continuous ambulatory peritoneal dialysis or
haemodialysis.
xi. Treatment (chosen for dosing convenience) of infections due to $\beta$-lactam susceptible Gram positive microorganisms in patients with renal failure.

xii. Use of vancomycin solution for topical application or irrigation.

Use vancomycin in health care centres increased appreciably after 1980s. The uses of third generation cephalosporins and anti-anaerobic agents have been recognized as a risk factor for colonization and infections with VRE. Other measures that are suggested for the control of VRE outbreaks include formulary policies discouraging the use of third generation cephalosporins and agents most likely to cause *C. difficile* colitis.\(^{[27,115]}\)

### ii. Education Programs

We should conduct Continuing educational programs for all hospital staff (including doctors, nurses, medical residents, students, technicians, pharmacists and patient caregivers etc. and include information about the epidemiology of VRE and the potential impact of this pathogen on the cost and outcome of patient care\(^{[115]}\) to create special awareness among them.\(^{[27]}\)

### iii. Role of Microbiology Laboratory for Detection, Reporting and Control of VRE:

Early detection of patients colonized or infected with VRE is an essential component of any hospital program designed to prevent nosocomial transmission of VRE.\(^{[19]}\) If the prevalence of VRE become high within an institution; prevention of transmission is very complicate. In the hospital, the microbiology laboratory plays an important role in the defence against the spread of VRE. The ability of the laboratory to identify enterococci and to detect vancomycin resistance promptly and accurately is essential in recognizing VRE colonization and infection and avoiding complication, costly containment efforts that are required when recognition of the problem has delayed. In many hospitals, the first case of VRE was detected by isolating VRE from clinical specimens submitted to the laboratory for clinical purposes. Even though few laboratories continue to perform antimicrobial susceptibility testing of enterococci only if they isolated from normally sterile body sites such as blood or urine, this practice is...
not at all appropriate for areas in which VRE have been encountered. Accordingly, once VRE are detected in a hospital, enterococci recovered from all body sites should be
tested for susceptibility to vancomycin. Hospitals that have not yet detected VRE but are located in (or receive patients from) geographic areas where VRE have been encountered should strongly consider performing susceptibility tests on all enterococcal isolates.\textsuperscript{[27]}

When VRE are isolated from a clinical specimen, it should be confirmed by repeating an antimicrobial susceptibility testing by recommended methods, particularly when VRE isolates are unusual in the hospital. While performing confirmatory susceptibility tests, the patient’s primary caregiver, patient care personnel on the ward in which the hospitalized patient is placed and infection control personnel must be immediately notified about the presumptive identification of VRE, so that the patient can be placed on appropriate isolation precautions promptly. This preliminary report must be followed by the result of the confirmatory test.

Enterococci may be tested for vancomycin resistance by using PCR assays designed to detect the genes responsible for glycopeptide resistance in these organisms. It is useful to speciate vancomycin resistant enterococcal isolates; such tests may be particularly helpful in part to distinguish the VanB and VanC organisms with low level resistance to vancomycin. Because VanC organisms are intrinsically resistant to vancomycin and resistance is non-transferable, isolation of patients found to be colonizing or infected with \textit{E. gallinarum} and \textit{E. casseliflavus} do not thought to be required. This distinction has implications for treatment and infection control.\textsuperscript{[27,28]}

Testing VRE isolates for susceptibility to teicoplanin by using simple disk diffusion tests will differentiate between VanA (teicoplanin resistant) and VanB (teicoplanin susceptible) strains in most instances. However, occasional teicoplanin-resistant VanB type strains have reported. Surveillance cultures for VRE are lengthy and costly for the laboratory. Some hospitals use PCR, it is rapid, effective and alternative to surveillance cultures.\textsuperscript{[27]}

The emergence of VRE, VRSA have surprised the global infectious diseases community.\textsuperscript{[19]} The clinical significant of VRE isolates is that they should be tested for susceptibility to as many as potentially active and commercially available drugs (e.g. ampicillin, quinupristin-dalfopristin, linezolid, chloramphenicol, tetracycline, fluoroquinolone and for urinary isolates, nitrofurantoin and possibly fosfomycin). When teicoplanin is available for treatment, it should be tested. \textit{E. faecalis} isolates were
causing major infections so they should be tested for $\beta$-lactamase activity by recommended method. Enterococcal isolates from patients with endocarditis, meningitis and isolates from sterile spaces, should undergo screening for high level aminoglycoside resistance.\textsuperscript{[28]}

In a hospital, the infections (out breaks) caused by single strain or by the simultaneous appearance of several different clones, are also important because this can affect the control measures. Various molecular typing methods have been used to establish the degree of clonal relatedness of VRE, including ribotyping, plasmid analysis, PFGE,\textsuperscript{[144]} arbitrarily primed PCR and examination of hybridization patterns obtained with probes, such as insertion sequences (IS6770) or \textit{vanA} and \textit{vanB} gene probes. A combination of genotypic methods such as PCR,\textsuperscript{[127,158]} PFGE plus plasmid analysis is likely to yield the most accurate information about the number of strains present in an institution and the patterns of transmission.\textsuperscript{[27]}

\section*{iv. Implementation of Infection Control Measures}

The enterococci are very tolerant organism and can survive on the hands of hospital personnel and surfaces of different equipments used in the hospitals.\textsuperscript{[22]} The current isolation precautions recommended by HICPAC to prevent patient-to-patient transmission of VRE are as follows.\textsuperscript{[27]}

i. Place VRE colonized or infected patients in single rooms or in the same room as other patients with VRE.

ii. When entering the room of a VRE colonized or infected patient we should wear clean non-sterile gloves. During the course of caring a patient, a change of gloves may be necessary after contact with material that may contain high number of VRE (e.g. stool).

iii. Wear a clean unsterile gown when entering the room of a VRE colonized or infected patient if substantial contact with the patient or environmental surfaces in the patient’s room is anticipated or if the patient is incontinent or has diarrhoea, an ileostomy, a colostomy or wound drainage not contained by a dressing.

iv. Remove gloves and gowns before leaving the patient’s room and wash hands immediately with an antiseptic soap or use a waterless antiseptic agent. Hands can
contaminate via glove leaks or during glove removal and bland soap is relatively ineffective in removing VRE from the hands.

v. Ensure that after glove and gown removal and hand washing, clothing and hands do not contact environmental surfaces potentially contaminated with VRE (e.g. doorknob or curtain) in the patient’s room.

vi. In some hospitals, it is required that gowns and gloves be worn routinely by all personnel entering a VRE patient’s room (Although it is not recommended by HICPAC).

Anecdotal experience in an ICU in which outbreaks caused by a single clone of VRE occurred revealed that routinely wearing gowns and gloves [115] before entering the rooms of patients, combined with other infection control measures, was effective in terminating the outbreaks. The fact that routine use of gowns did not seem to provide additional protection against VRE transmission, but it may causes heavy environmental contamination. Further studies will be needed to establish the circumstances in which routine use of gowns by personnel provides additional protection against the spread of VRE. [27]

In addition to these isolation precautions, the use of noncritical items such as stethoscopes, sphygmomanometers or rectal thermometers should dedicated to a single patient or cohort of patients colonized or infected with VRE. Culture of stools or rectal swabs of roommates of patients newly found to be colonized or infected with VRE must be performed to determine the colonization status and to see whether isolation precautions are necessary. Additional screening of patients on the ward can be performed at the discretion of the infection control staff. A policy decision when patients colonized and/or infected with VRE can be removed from isolation precautions should be adopted. However, since VRE colonization may continue forever, rigid criteria may be suitable, e.g. VRE negative cultures on at least three consecutive occasions, one week or more apart, for all cultures from multiple body sites (including stool, rectal swab, perineal area, axilla, umbilicus, wound, Foley catheter and/or colostomy sites if present). A system of highlighting the records of colonized or infected patients should be established so that they can be recognized and placed on isolation precautions promptly upon readmission to the hospital, because patients with VRE may remain colonized for long periods following discharge from the hospital. Ideally, this information should be computerized so that placement of colonized patients will not delay due to unavailability of the patient’s medical records. Local and state
health departments should be consulted in developing a plan for the discharge of VRE colonized or infected patients to nursing homes, other hospitals, or home health care as part of a larger strategy for handling patients with resolving infections and patients colonized with antimicrobial resistant microorganisms.\textsuperscript{[27]}

The HICPAC has some extra recommendations for hospitals where endemic VRE or VRE transmission continued in spite of the implementation of above mentioned measures. These are as follows.\textsuperscript{[27]}

i. Control efforts should be initially focused in ICUs and on areas where the VRE transmission rate is highest. Such units may serve as a reservoir for VRE, from which VRE spreads to other wards when patients are well enough to transfer.

ii. Where feasible, staffs that provide regular care to patients should be cohorted to minimize the movement of health care givers between VRE positive and VRE negative patients.

iii. In conjunction with careful epidemiologic studies and upon the direction of the infection control staff, personnel should be examined for chronic skin and nail problems. Hand and rectal swab cultures should be performed on specimens obtained from them. VRE-positive personnel epidemiologically linked to VRE transmission should be removed from the care of VRE-negative patients until their carrier state is eradicated.

iv. The results of several enterococcal outbreak investigations suggest a potential role of the environment in the transmission of enterococci. Institutions experiencing ongoing VRE transmission should verify that the hospital has adequate procedures for the routine care, cleaning and disinfection of environmental surfaces (e.g. bedrails, charts, carts, doorknobs, faucet handles and bedside commodes) and that these procedures are being followed by housekeeping personnel. Some hospitals may be selected to perform focused environmental cultures before and after cleaning rooms (housing patients with VRE) to verify the efficacy of hospital policies and procedures. All environmental culture should be approved and supervised by the infection control program in collaboration with the clinical laboratory.

v. Representative VRE isolates should be sent to reference laboratories for strain typing by PFGE or other suitable techniques to aid in defining reservoirs and patterns of transmission.
Some hospitals are slow to implement the new HICPAC recommendations for isolation of patients with VRE and continue to use more traditional isolation systems such as “contact isolation” (initially described in 1983) or “body substance isolation”. Unfortunately, numbers of VRE outbreaks have continued despite the use of these policies. Therefore, all hospitals should strictly implemented barrier precautions recommended by HICPAC and continuing doing so.\textsuperscript{[27]}

In the absence of infection control efforts VRE rates might have increased more. Unfortunately, breaches in infection control protocols occur commonly. Many studies have confirmed that fulfilment of health care workers with recommended barrier precautions is habitually. Accordingly, hospitals that have trouble in controlling the nosocomial transmission of VRE should consider developing systems for monitoring and improving the compliance of personnel with recommended barrier precautions.\textsuperscript{[27]}

**Control of VRE in Long-Term-Care Facilities (LTCFs)**

The colonized residents of LTCFs may provide as a reservoir of VRE for acute care. VRE may not be a common cause of infection in residents of LTCFs and yet not been reported as causative agent of serious illness in LTCF patients. The highly immune-compromised patients are probable victim for serious VRE infection.\textsuperscript{[27]}

The Committee believes that patients who are colonized or infected with VRE may be cared for safely in LTCF with minimal risk of development of nosocomial infection in other patients and that the task of carrier identification and proper isolation probably is borne most appropriately by acute care institutions. According to the recommendations of the Committee, the general approach to control of VRE in LTCFs is summarized as follows.\textsuperscript{[27]}

i. Employee education about basic infection control and VRE is essential to any effort to control these organisms.

ii. If an outbreak of infection appears to be under way, surveillance cultures of swabs of the rectum or perirectal area and wounds for VRE may be appropriate. Otherwise, they are unlikely to be cost effective and are not recommended.

iii. When a patient colonized or infected with VRE is transferred from LTCF to an acute care facility, this information should be provided to the receiving institution.
iv. As an initial recommendation, the Committee advises continuing VRE isolation until at least two rectal cultures (or wound cultures) taken on separate days are found to be negative for the organism.

v. If possible, patients colonized or infected with VRE should be placed in personal rooms. If the patient must share a room unavoidable circumstance, a roommate colonized with the same organism should be preferred. If there is no private room available, a patient with VRE (who is continent of stool, does not have diarrhoea, and does not have an open wound colonized or infected with VRE) can placed in the same room with another patient. Although, there is no data available, it would be appropriate that the other patient is not severely immunocompromised (i.e. not have organ transplantation, not neutropenic, not suffering from severe acute or chronic illness and not been treated recently with multiple or broad-spectrum antibiotics), not have open wounds, not be receiving antibiotics and not have an indwelling catheter or other drainage device. Similar precautions would be appropriate if colonized or infected patient must share a bathroom with another patient. Careful hand washing is necessary and caring for the non-colonized roommate before contact with the VRE colonized patient or his environment would be appropriate.

vi. Gloves (clean and nonsterile) are required before contact with a colonized or infected patient, his or her secretions, and inanimate environment within the room. Gloves should be changed after contact with materials that have a high concentration of microorganisms (e.g. stool) and before contact with the roommate or his or her immediate environment. Hands should be washed with an antiseptic agent containing chlorohexidine or alcohol after removing the gloves.

vii. Gowns are required if it is expected that the health care worker’s clothing will have material contact with the patient, patient’s secretions or environmental surfaces. Gowns are especially important if a patient has diarrhoea or a wound with drainage not contained in a dressing. Care must taken to avoid environmental contact by clothing after removing the gown. Gowns must be disposed of in a way that will minimize contamination of the environment.

viii. Patient transport should be limited to situations required for medical care and precautions must continue to prevent transmission to other patients and to prevent contamination of environmental surfaces. Room restrictions probably are
appropriate only for patients with wound drainage not contained in a dressing or for those who are incontinent or who have diarrhoea.

ix. Patient care equipment should be dedicated to a single patient only. If this is impossible, appropriate cleaning and disinfection should be done between patients. The use of individual thermometers is strongly encouraged. Areas that the patient may contaminate like bedrails, bedside tables, commodes, soap dispensers, faucets, door handles, etc. should be cleaned frequently (at least daily) with an appropriate disinfectant.

x. Recommendations for prudent vancomycin use should follow.

**Surveillance Cultures**

Performing special surveillance cultures of patients to detect gastrointestinal colonization not identified by clinical cultures has been often proved useful during outbreaks and should considered an essential component of successful VRE control programs. The collapsed performance of prevalence surveys may consequence in poor control of VRE in hospitals. Prevalence surveys may be insufficient like culturing samples only from roommates of known VRE patients or extra comprehensive like culturing samples from all patients from a ward in which a recently revealed VRE case has occurred or obtaining periodic samples for culture from high risk patients such as those in ICUs, haematology-oncology wards or transplantation units. The strategies to identify colonized patients include screening stool specimens submitted for *C. difficile* toxin assays for VRE and screening rectal or perirectal swab specimens obtained from patients admitted from high risk institutions (hospitals and LTCFs in which VRE are endemic). Perirectal cultures seem to have sensitivity similar to rectal cultures for detecting colonized individuals.[27]

Surveillance cultures of stool and specimens from rectal or perirectal area should be inoculated onto vancomycin selective media. To confirm the vancomycin resistance, brain heart infusion agar containing vancomycin are found very useful for testing isolated colonies of enterococci but they are not suitable for screening of direct stool sample for VRE, because they support the growth of many other organisms.[27,28]
Elimination of Reservoirs

The environment surrounding the infected patients might be contaminated with enterococci and it may act as reservoir to allow continuous spread of organisms. A thorough cleaning of the environment is necessary to control an epidemic of VRE infection.[22]

Attempts to Eradicate Gastrointestinal Colonization

The eradication of resistant enterococci in human carriers is more problematic. Several studies have revealed that both patients and health care workers can harbour resistant enterococci in their gastrointestinal tract. During one outbreak of \( \beta \)-lactamase producing gentamicin resistant enterococci, a 14 days course of oral vancomycin and rifampicin with total body chlorohexidine scrubs was used to eradicate carriage in nurse. This antibiotic regimen was based on the isolate’s antibiotic susceptibilities.[22]

There has been interest in eradication of gastrointestinal colonization with VRE for the following reasons:[27]

i. To decrease the subsequent risk of infection in the individual patient,

ii. To minimize inconvenience to the patient and costs of hospital associated infection with infection control procedures applicable to colonized patients and

iii. To reduce the reservoir of VRE in the institutional environment.

Combinations of novobiocin with doxycycline or tetracycline failed to eradicate VRE from the stools of seven among eight treated patients. Two groups reported more promising results with oral bacitracin alone or with combination with doxycycline initially cleared VRE from the stools of all treated patients.[28] Although, some patients responded well to these antibiotics for decolonization but there is no uniformly effective regimen for eradicating VRE from gastrointestinal tract. Determination of whether VRE can be eradicated from the gastrointestinal tract by antimicrobial chemotherapy will require prolonged observation and use of enrichment medium (glycopeptides), to detect low densities of VRE remaining in the stool, before considering any regimen for therapy.[27] It is unknown what will emerge as the optimal method to eliminate carriage of enterococci in both patients and hospital personnel.