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Enterococcus: An Emerging Global Superbug

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Abstract: Enterococci are part of the normal intestinal flora of humans and animals but are also important pathogens responsible for health care-associated and community-onset infections. These infections constitute a major problem in terms of medical and socio-economic costs and cause significant morbidity and mortality. Of late, they are among the most frequent clinical isolates in intensive care units (ICUs). With increasing antibiotic resistance, Enterococci are recognized as feared nosocomial pathogens that can be challenging to treat.

Keywords: Enterococcus, enterococci, hospital acquired infection, nosocomial infection, superbug

1. Introduction

Enterococci, posses Lancefield group D antigen and were formally grouped as faecal streptococci. They are now separated from Non-enterococcal group D streptococci and have been reclassified in to a separate genus Enterococcus [1]**.** They are natural resident of human and animal gastrointestinal tract. They continuously grow and multiply harmlessly in the nutritionally rich intestinal content without causing disease in the host. However, if the individual becomes immunocompromised, these bacteria tend to cause serious infections. They are frequently isolated in large number from faeces of otherwise healthy individuals. Most commonly isolated species from clinical samples are *Enterococcus fecalis* and *Enterococcus faecium*. Other species known to cause human infections include *E. avium, E. gallinarum, E. casseliflavus, E. durans, E. disper, E. raffinosus, E. malordaratus, E. mundtii and E. flavescens [2],[3]*.

2. Microbiology

Enterococci are gram positive ovoid shaped cocci mostly appear in pairs with cells arranged at an angle to each other but also in short chains. They are aerobic and facultative anaerobic organisms that can grow over a wide range of temperature, 10-45ºC. They produce tiny dark red magenta colonies on MacConkey agar and small yellow colonies on CLED (cysteine lactose electrolyte-deficient) agar. They are usually non-haemolytic, though some strains may show alpha or beta haemolysis [4]**.** They also produce small black colonies on Tellurite-Blood agar. They have distinctive features that differentiate them from other streptococci. These distinctive features include their ability to grow in 40% bile, 6.5% sodium chloride at pH 9.6 at 45ºC and in 0.1 % methylene blue milk [5]. They can survive 60ºC for 30 minutes. They are generally non-motile, non capsulated, non-sporing, non pigment producing bacteria. However, few species deviates from this general rule. Yellowish pigment is produced by *E. casseliflavus, E. mundtii* and *E. flavescens*. While motility is observed in *E. gallinarun* as well as *E. casseliflavus*. Enterococcal biochemical activities form the ground for their identification. They hydrolyse aesculin in the presence of bile in to aesculatin, producing black precipitate in bile aesculin agar. They also hydrolyse L-pyrrolidonyl-β-naphylamide, producing red colour product (positive PYRase test). They ferment lactose (mannitol and sorbitol) with acid and no gas production. They also reduce (decolourise) litmus milk, producing white to pale yellow colour [1], [4], [6]. Individual species can be identified by their motility, pigment production and typical biochemical reactions especially fermentation of specific sugars [6]. For example, *E. fecalis* can be identified by its ability to ferment mannitol, pyruvate, sorbitol and its inability to ferment arabinose. While E. *fecium* can be identified by its ability to ferment arabinose, mannitol and its inability to ferment Pyruvate [1].

3. Virulence and Pathogenicity

The ability to acquire and spread genes responsible for antimicrobial resistance is the main reason for enterococci emerging as important nosocomial pathogens. However, other virulence factors are equally important in disease causation by these organisms. Factors such as biofilm formation, gelatinase and haemolysin production contribute immensely in disease causing processes of enterococcal infections. Adherence to body surfaces is considered a major factor responsible for the pathogenicity of the clinical enterococcal isolates. Strains causing infection are considered to have a greater capacity to adhere to surfaces. The enterococcal surface protein (esp) plays a major role in the capability of enterococcal strains to form biofilms [7], [8]. "esp" was first described in a virulent gentamicin-resistant *Enterococcus faecalis* isolate. Biofilm formation plays a major role in nosocomial infections like catheter-associated UTIs, blood stream infections due to pacemakers, artificial heart valves, artificial hip prosthesis etc., and this capability to produce biofilms has been considered an important virulence factor of these organisms [9]. Various methods like microscopic biofilm formation assay and epifluorescence microscopy have been tried to study the biofilm-forming capability of the bacteria. However, the method that has been used very frequently in recent times is the microtiter plate biofilm production assay. This method is preferred because of its simplicity and cost-effectiveness [8].

Gelatinase elaborated by some *Enterococcus* isolates has been identified to be an extracellular zinc-endopeptidase capable of hydrolyzing gelatin, collagen, casein, hemoglobin and other peptides. This virulence factor is mostly seen in isolates of *E. faecalis*. The role of gelatinase in enterococcal infections is to provide nutrition to the bacteria by degradation of host tissues [10], [11].

Hemolysins (cytolysins) have been shown to be associated with increased mortality in experimental animals. Cytolysin genes is mainly found on the plasmid and have also shown to be associated with bacteriocin (Enterocin) production with activity against some gram-positive such as *Listeria species*, *Clostridium species* and even *Staphylococcus aureus* [8].

Other virulence factors identified include collagen binding protein, cell wall adhension protein, extracytoplasmic function sigma (SigV) factor and capsular polysaccharide (CpsF) production [10], [12].

4. Spectrum of Infections Caused by Enterococci

Though commensal in adult faeces, enterococci are important pathogens especially in hospital environment. Historically, enterococci as human pathogens was confirmed first in 1912, by Hicks and subsequent workers [6]. Though Thiercelin described Enterocoque associated with enteritis, appendicitis and menningitis in 1899 and also *Micrococcus zymogenes* (which was later thought to be *Streptococcus faecalis* var. *zymogenes*) was isolated from endocarditic patient in same year. Similarly Andrew and Horder isolated *Streptococcus faecalis* from patient with endocarditis and UTI in 1906 [6].

Enterococcus has been implicated with endocarditis for the past few decades; however, overtime, there has been a worldwide increase in the spectrum of enterococcal infections and emergence of antibiotic resistance among the clinical isolates, especially in hospital settings [13]. The most common infection caused by enterococci is urinary tract infections (UTIs), followed by intra-abodominal or intra-pelvic abscess as well as surgical wound infections. Blood stream infections are also ranked up among the enterococcal infections [14]. Other infections associated with *Enterococcus* include neonatal infections, central nervous system (CNS) infections, osteomyelitis, cholecystitis, respiratory tract infections and dental infections [6], [13]. Epidemiological studies in United States have reported that Enterococci are the second most common cause of nosocomial infections, third most common cause of nosocomial bacteraemia and also important causes of community-acquired infections (CAIs) [15]. They are among the most frequent clinical isolates in intensive care units (ICUs) [16], [17].

The distribution of *Enterococcus species* varies throughout Europe. In UK, there were 7066 reported cases of bacteraemia caused by *Enterococcus* species in 2005. Twenty-eight per cent of those cases were antibiotic resistant. In Spain and the UK combined, E. *faecalis* and *E. faecium* are the most commonly isolated species from both clinical and environmental sources [18].

In Nigeria, the prevalence rate of hospital-acquired enterococcal infections was 5.9% of which 85.7 % was due to *Enterococcus faecalis and 14.3% due to Enterococcus faecium* [19].

Similarly in India, the most common species isolated was *E. faecalis* (64-87%), followed by *E. faecium* (10-32%) and *E. durans* (2.05%) [20], [21]. In Mumbai, clinical isolates from tertiary hospital revealed 70.1% and 29.9% due to *E. faecalis* and *E faecium* respectively [22]. Saraswathy *et al*. [23] found that 87.5% of enterococcal isolates were *E. faecalis* and 8.9% were *E. faecium*, in Tamil Nadu.

So many species have been linked with human infections but the most common isolate from clinical samples are *E. faecalis* and *E. fecium*. While *E. faecalis* remains the predominant species, *E. fecium* isolates are increasing in proportion especially from blood samples. Furthermore, enterococcal resistance against major groups of antibiotics such as β-lactam antibiotics, aminoglycosides and glycopeptides including vancomycin is high, and increasing, making treatment of enterococcal infections more challenging to clinicians [13], [14], [24].

5. Antibiotic Resistance

Emergence of resistance to wide range of antibiotics is the most important feature of enterococci as the cause of hospital acquired infections. They demonstrate both intrinsic and acquired resistance [6], [13], [25].

5.1 Intrinsic Resistance

Enterococci are intrinsically resistant to most beta-lactam antibiotics because of low affinity penicillin binding proteins (PBPs), which enable them to synthesize cell wall components even in the presence of modest concentration of most β-lactam antibiotics (for example, at minimum inhibitory concentrations (MICs) of penicillin that generally range from 1-8 µg/mL for *E. faecalis* and 16-64 µg/mL for *E. faecium*). This makes them "tolerant" to the activity of β-lactam antibiotics, in which they are only inhibited but not killed by them [14], [25], [26]. They also exhibit a low to moderate level resistance to aminoglycosides (MIC 62-500 µg/mL) due to the slow uptake or permeability of these agents. However, aminoglycoside uptake can be enhanced when enterococci are exposed to a β-lactam (which increases the intracellular uptake) [24]. Enteroccci also exhibit intrinsic low level resistance against clindamycin, lincomycin and trimethoprin-sulfamethoxazole (due to their ability to utilize exogenous source of folate)[6]. Being Gram-positive bacteria, enterococci are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid [27].

5.2 Acquired Resistance

Acquired resistance can either be via mutations of exiting DNA or through acquisition of new DNA. High level resistance (HLR) is usually acquired via plasmid mediated production of aminoglycoside-inactivating enzymes. However, some ribosomally mediated mechanisms have also been noted. The enzymes produced include streptomycin adenyltranferase which confers resistance to streptomycin and a bifunctional enzyme that possesses 6' acetyltransferase and 2' phosphortranferase activities posing resistance against all aminoglycosides except streptomycin [13]. The first transferable resistance among enterococci was chloramphenicol resistance which has been mediated by

chloramphenicol acetyl tranferase. Later erythromycin resistance and HLR to clindamycin were demonstrated as part of macrolide-lincosamide-streptogramin B resistance phenotype which involves methylation of adenosine residue in 23S rRNA [6]. Active efflux of tetracycline mediated by *tetL* gene is the main mechanism associated with enterococcal tetracycline resistance [24]. Enterococci also produce β-lactamase enzyme that hydrolyses penicillin, ampicillin, and piperacillin. Beta-lactamase production is sometimes plasmid mediated and may be associated with high-level gentamicin resistance. Enterococcal resistance to beta-lactam antibiotics is low-level and is not detected by routine disk susceptibility testing due to "*inoculum effect"*. This is because low number of cells do not produce sufficient β-lactamase to show resistance. Unlike that from staphylococci, the β-lactamase from enterococci is constitutively produced and cell-bound [26]. Recommended and reliable β-lactaamse detection is by use of chromogenic cephalosporin, nitrocefin [1].

5.2.1 *High level aminoglycoside resistance*

Aminoglycoside resistance of MIC >2000µg/mL and MIC >500µg/mL for streptomycin and gentamycin respectively is defined as high level resistance [27]. Though enterococci typically have intrinsic low-level resistance to aminoglycosides, high level aminoglycoside resistance in enterococci is predominantly mediated by transferable plasmid mediated enzymes. such enzyme include a bifunctional aminoglycoside modifying enzyme 2'phosphotransferase and 6'acetyl transferase conferring HLR to all available aminoglycosides (kanamycin, gentamicin, amikacin, netilmicin, tobramycin) except streptomycin [26]. The action of this enzyme in enterococci eliminates the synergistic activity of aminoglycosides when combined with a cell wall active agent, such as ampicillin or vancomycin [27], [28].

Some strains show HLR to amikacin and kanamycin without HLR to any other aminoglycosides. This is due to production of aminoglycoside 3' phosphotransferase enzyme [13]. In addition to plasmid mediated resistance, streptomycin resistance is mediated ribosomally. It appears that hydroxyl group at position 6 of the antibiotic is being adenylylated by streptomycin 6' adenyltranferase enzyme conferring HLR to streptomycin but does not inactivate other aminoglycosides [6], [26].

Screening for HLAR

Routine disk diffusion does not detect HLAR. High content disc such as 120µg of gentamicin or kanamycin and 300µ of streptomycin are recommended. Resistance is indicated by zone of 6mm while susceptibility by zone of ≥10mm and zone between 7mm to 9mm is inconclusive. Inconclusive results need to be confirmed by agar dilution or broth microdilution test [27].

5.2.2 Glycopeptides resistance

Glycopepetides are potent cell wall synthesis inhibitors. They bind the D-alanyl-D-alanine portion of the peptidoglycan side chain precursor where by making it in accessible to PBPs. In Vancomycin Resistant Enterococci (VRE), the D-alanyl–D-alanine dipeptide is changed, most often to D-alanyl–D-lactate. Glycopeptides are no longer able to recognize and bind to these altered precursors. It became clear that development of glycopeptides resistance is the major achievement by these organisms. Unfortunately, the gene clusters that encode this activity in enterococci are transferable and have already been found in *S. aureus*. Appearance of VRE was first reported in Europe in mid1980s. Initially described in United Kingdom, France, Germany and Spain and later spread rapidly to other part of the world [13], [14]. There has been a genetic linkage in *E. fecium* between ampicillin, and vancomycin resistance [14].

Newer antibiotics (eg, quinupristin-dalfopristin, linezolid, daptomycin, tigecycline) with activity against many VRE strains have improved this situation, but resistance to these agents has already been described. A mutation (G2576U) in the domain V of the 23S rRNA is responsible for linezolid resistance, whereas resistance to quinupristin-dalfopristin may be the result of several mechanisms: modification of enzymes, active efflux, and target modification. Resistance of *E. faecalis* and *E. faecium* to daptomycin, a newer cyclic lipopeptide antibiotic that acts on the bacterial cell membrane, has also been reported [25].

At least six phenotypes of vancomycin resistance, termed VanA, VanB, VanC, VanD, VanE, and VanG, have been described so far. The VanA and VanB phenotypes are most clinically significant. The details of vancomycin resistance have been best documented with the *vanA* gene cluster found on the transposon, or "jumping" genetic element, *Tn1546* [13], [26].

There are 3 major phenotypes seen in Europe and United states which include VanA, VanB, and VanD. VanA is the most common (70% to 80%), and enterococcal isolates exhibit inducible, high-level resistance to both vancomycin (MICs ≥64µg/mL) and teicoplanin (MICs ≥16µg/mL), while VanB isolates (up to about 20%) have inducible variable resistance to vancomycin (MICs 32- 64µg/mL) and remain susceptible to teicoplanin [13], [14], [26]. The VanC phenotype is mediated by the chromosomal *VANC1* and *VANC2* genes, which demonstrate intrinsic, low-level reistance to vancomycin (MICs 2 - 32µg/mL) and are susceptible to teicoplanin and is limited to *E. gallinarum* (*VANC1*), *E. casseliflavus* (*VANC2*), and *E. flavescens*. To date, the VanD, VanE, and VanG phenotypes have been described in only a few strains of enterococci [13], [25]. There is a marked difference in the prevalence of vancomycin resistance in species of Enterococci. In US, *E. faecalis* accounts for 3% to 5% of VRE, while up to 46% of VRE have been shown to be *E. faecium* [14].

6. Treatment of enterococcal infections

A truly remarkable aspect of the enterococci is their resistance to many antibiotics and their potential for acquiring and disseminating resistant genes makes treatment of enterococcal infections clinically challenging. Despite that, some therapeutic options are worthy of mention due to their clinically proven positive outcomes.

Prior to treatment of enterococcal infections, all suspected intravenous lines, intra-arterial catheters, and urinary catheters should be removed, if possible, and abscesses

drained. Treatment of uncomplicatated enterococcal infections (such as UTIs, Intra-abdominal infections, uncomplicated wound infections etc) is limited to monotherapy such as penicillins, in particular penicillin G, ampicillin and piperacillin. Unfortunately, enterococci are increasingly resistant to these antibiotics, often because of production of altered PBPs that do not bind β-lactams. In such strains, vancomycin is used in place of β-lactam antibiotics. However, relapse or primary failure occurs as penicillin or ampicillin or vancomycin alone produces a bacteriostatic rather than bactericidal effect [13], [24], [26].

Serious enterococcal infections (*e.g.*, bacteraemia, endocarditis, meningitis, and osteomyelitis) require treatment with a bactericidal combination of antibiotics that should include penicillin (ampicillin or penicillin G) to which the isolate is susceptible and an aminoglycoside (gentamicin or streptomycin) to which it does not shows high-level resistance [25], [29]. Vancomycin can also be used in combination with an aminoglycoside, and is recommended as a drug of choice in patients with serious penicillin allergy or in treatment of infections caused by penicillinase producing strains. Resistance to vancomycin, however, has also become common [30]. Teicoplanin can be used in patients exhibiting the Van B phenotype preferably in combination with streptomycin or gentamicin (if not resistant). Unless penicillin susceptible, VRE must be treated with linezolid, daptomycin, tigecycline, or quinupristin/dalfopristin. It has been demonstarted that quinupristin/dalfopristin is only active against *E. faecium*, but not *E. faecalis* [24]. It should be noted that the choice of antibiotics should not only depend on antimicrobial susceptibility results, but also on the type of infection being treated (endocarditis versus urinary tract infection), the severity of this infection and clinical response to the regimen chosen [26].

7. Conclusion

It is important to recognize enterococci as significant pathogen for their remarkable tendency to acquire resistance to major groups of antibiotics. Severe enterococcal infections pose serious challenge to treatment, making enterococci important pathogen especially in critical care units. Their ability to tenaciously remain in hospital environment and to transfer resistant gene to other microorganisms constitutes a great threat to hospital infection control.

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