Chapter

Enterococci: An Important Nosocomial Pathogen

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Abstract

Enterococci, particularly *Enterococcus faecalis* and *Enterococcus faecium*, are an important cause of nosocomial infections and have become a major issue worldwide. Nosocomial infections due to vancomycin resistant Enterococci (VRE) occur frequently. A significant increase in prevalence of VRE has been reported recently in many countries. Enterococci are second most frequent cause of nosocomial urinary tract infection, bacteremia and infective endocarditis. They are also related to etiology of intra-abdominal an pelvic infections, gastrointestinal infections and oral infections. The ability of Enterococci to survive in adverse conditions, presence of virulence factors and possession of intrinsic and acquired antibiotic resistance traits poses a therapeutic challenge. Due to high level of multidrug resistance in VRE, Enterococcus has become an important organism in health based settings.

Keywords: Enterococci, vancomycin, resistance, nosocomial, infections

1. Introduction

Enterococci are Gram-positive, non-spore forming and facultative anaerobic cocci. They are indigenous flora of the intestinal tract, oral cavity and vagina in healthy persons. The genus comprises 54 species which are ubiquitously present in nature [1]. Enterococci have emerged as an important nosocomial pathogens second to Staphylococci which is the leading cause of nosocomial infections worldwide [2]. Enterococci are important nosocomial pathogens causing up to 10% of all infections in the hospitalized patients [3]. In these Enterococci infections approximately 60% of infections are caused by *Enterococcus faecalis* and *Enterococcus faecium* causes the remaining [4]. In the last decade both *E. faecalis* and *E. faecium* have emerged as important nosocomial pathogens. Other Enterococcal species causing nosocomial human infections are *E. avium*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. raffinosus* and *E. mundtii*. Majority of clinical isolates (63–81%) are identified as *E. faecalis*, around 13–23% as *E. faecium* and other enterococcal species comprise around 3–4% of the clinical isolates [5].

2. Prevalence of vancomycin resistant Enterococci (VRE)

Nosocomial infections particularly by vancomycin resistant Enterococci (VRE) have become a major problem since last few years though VRE are organisms of low virulence and pathogenicity. Nosocomial infections caused by VRE are highly

prevalent in intensive care units of hospitals. These infections are particularly high in presence of underlying health factors like diabetes, liver transplantation, neutropenia, diabetes mellitus and renal dysfunction. Recently it has also been seen that VRE bloodstream infections have higher mortality rates as compared to vancomycin susceptible Enterococci (VSE) [6, 7]. Data from countries like Germany shows an increase of VRE from less than 5% in 2001 to 14.5% in 2013 mainly vancomycin resistant *E. faecium* [8]. In Europe of all the nosocomial infections reported 9.6% were of Enterococci [9]. In USA 3% of the nosocomial infections are due to VRE [10]. VRE nosocomial infections cause greater number of invasive treatment resulting in extended stay in hospital and cost [11]. Hospitals in some countries have now established VRE screening in high risk areas and isolation of patients to prevent spread of the resistant pathogen [12]. A study has shown the prevalence of VRE colonization in patients who had history of previous administration of antibiotics for more than 2 weeks were 10 times more likely of getting VRE colonization [13]. Other studies have also reported similar findings which show antibiotic exposure can cause colonization of VRE in hospital settings because of their resistance to commonly used antibiotics, virulence factors and ability to acquire genes [14].

3. Genetic factors and antibiotic resistance in VRE

The genes Van A, Van B, Van C, Van D and Van E are responsible for vancomycin resistance in Enterococci. Van M has been identified which is also an important vancomycin resistant determinant among different *E. faecium* lineages in hospitals in Shanghai, China [15]. Each Van operon has different ecological origin, Van A has originated from soil organisms, van B, Van G and Van D from gut microbiota [16]. Vancomycin resistance in Enterococci is of two types (a) Intrinsic resistance—Enterococci spp. like *E. gallinarum* and *E. casseliflavus* show an inherent low level resistance to Vancomycin. They have Van C genes that produce Vancomycin minimum inhibitory concentration (2–32 μg/ml) [17] A hospital wide outbreak of vancomycin resistant *E. gallinarum* has been reported in Colombia showing that uncommon species of Enterococci are capable of spreading in the hospital environment and producing nosocomial infections [18]. The second type is (b) acquired resistance—Enterococci species acquire resistance genes and become resistant to vancomycin. This is seen in *E. faecium* and *E. faecalis* and to some extent in *E. raffinosus*, *E. avium*, *E. durance* and other enterococcal species. The most common isolated Enterococci species which is VRE in hospital settings is *E. faecium*. It has been seen that *E. faecium* produces high vancomycin minimum inhibitory concentration (64–1000 μg/ml) [19]. There has been a significant increase in VRE prevalence. The emergence and rapid spread of VRE has led to the use of new antibiotics like linezolid, daptomycin and tigecycline. Linezolid is a oxazolidinone antibiotic. An oxazolidinone resistance gene optr A has been identified in *E. faecalis* and *E. faecium* isolates of human and animal origin [20] .Linezolid resistance is still less prevalent reported as 1.1 and 1.8% in *E. faecium* and *E. faecalis* isolates from 19 US hospitals [21]. Daptomycin resistance is more prevalent in *E. faecium* than *E. faecalis* isolates. Around 3.9 and 0.2% of *E. faecium* and *E. faecalis* isolates have been reported in various hospital settings [22]. Tigecycline is a semisynthetic derivative of tetracycline. Tigecycline resistance in *E. faecium* and *E. faecalis* is rare and reported as 0.3%. It is being used to treat bacteremia caused by MDR enterococci. The increased use of antibiotics in hospitals is causing gut dysbiosis and enterococci possess surviving ability take over the niche in the gastrointestinal tract and this could be the primary source of enterococcal infections [23].

4. Lineages of nosocomial Enterococci

The ability of *E. faecium* to exchange mobile genetic elements carrying antimicrobial resistance genes and virulence determinants has resulted in hospital adapted clones [24]. Esp was the first adaptive element found in hospital strains of *E. faecium*. The *E. faecium* esp. gene has been linked to biofilm formation, UTI and endocarditis [24]. New determinants have been now linked to hospital isolates of *E. faecium*. A genomic analysis study of *E. faecium* hospital strains identified gain and loss of gene clusters in clinical and non-clinical isolates of *E. faecium* [25]. Genomic studies of nosocomial *E. faecium* infection have confirmed the transmission of *E. faecium* Clad A115. Recently it has been seen a significant presence of hospital associated VRE fm lineages in the wastewater and need of controlling healthcare associated dissemination of VRE fm [26]. However studies on *E. faecalis* ecotypes have shown no appearance of distinct *E. faecalis* strains over a significant period of time. Virulence factors like antibiotic resistance and virulence genes, esp., capsule polysaccharide genes and genes determining gelatinase, aggregation factor, cytolysin and ace are identified in *E. faecalis* isolates [27]. The non-emergence of distinct ecotypes of *E. faecalis* and multiplicity of closely related ecotypes is not seen in *E. faecalis* as compared to *E. faecium*. A genomic analysis of 168 *E. faecalis* hospital isolates showed no genes and non-synonymous single nucleotide polymorphisms in the three lineages of hospital strains [28]. A recent study has also demonstrated that the acquisition of mobile genetic elements in *E. faecalis* V583, makes it unable to coexist with commensal enterococci in humans [29].

5. Nosocomial infections by VRE

Nosocomial infections by Enterococci are Urinary tract infection, endocarditis, bacteremia, catheter related infections, wound infections, intra- abdominal and pelvic infections and recently even oral infections have been reported.

6. Urinary tract infection (UTI)

Enterococci cause both uncomplicated and complicated health care associated UTI. *E. faecalis*. Vancomycin resistant *E. faecalis* and vancomycin resistant *E. faecium* have been mainly implicated in Enterococcal UTI. VRE is fast becoming a major cause of health care associated UTI. The treatment of UTI involves the use of broad spectrum antibiotics which is a major cause of resistant strains to vancomycin (VRE). The complications range from uncomplicated cystitis, pyelonephritis, perinephric abscess, and prostatitis. These organisms are responsible for nosocomial infection of urinary tract particularly in intensive care units (ICU). Enterococci have been particularly reported in catheter associated urinary tract infections, CAUTI (28.4%). Enterococci species are capable of producing biofilms, which are a population of cells attached irreversibly on various biotic and abiotic surfaces. CAUTI are associated with multispecies biofilms. Biofilms are difficult to remove and result in many chronic infections. Bacteria in biofilms colonize medical devices such as catheters, pacemakers, prosthetic heart valves and orthopedic appliances [30]. These multispecies biofilms have synergistic or antagonistic effects of interspecies interaction. Many studies have shown the association of biofilm producing enterococci and urinary catheter [31, 32]. Enterococci biofilms which are formed on catheter in CAUTI are resistant to immune clearance, urination

force and even antibiotics. These enterococci utilize fibrinogen formed on catheter surface and form resistant biofilms. *E. faecalis* attachment in biofilm formation seen in vitro is partially inhibited by uropathogenic *E. coli* (UPEC) but biofilm formation by *K. pneumoniae* or UPEC are not affected by *E. faecalis* but *E. faecalis* increased *E. coli* biofilm mass accumulation and it has been seen that co-culture of an *E. faecium* probiotic strain with enteropathogenic *E. coli* increased the antibiotic sensitivity of *E. coli* to aminoglycosides, B-lactams and quinolones [33]. Biofilm formation confers the organism resistance to phagocytosis and antimicrobial agents. UTI by *E. faecalis* is mediated by virulence factors of the genes esp., srtC, ebp A, ebpC, ace, epaB, msrA, msr B, sigV, efbA, and grvR/etaR. *E. faecium* also displays similar genes related to virulence. Both *E. faecalis* and *E. faecium* isolated from nosocomial UTIs show kidney tropism. It is important to study factors in enterococcal causing pyelonephritis [33].

7. Bacteremia

There is a high prevalence of blood stream infections caused by Gram-positive bacteria and 45% are caused by Enterococci. Bacteremia is a common manifestation of vancomycin resistant Enterococci. Due to use of intravascular and urinary catheters these nosocomial infections are acquired. *E. faecium* in the blood stream is associated with increased mortality due to high levels of resistance. Risk factors identified with VRE bacteremia include intestinal colonization, long term antibiotic use, severity of illness, bone marrow transplant, hematologic malignancy, indwelling urinary catheters, corticosteroid treatment, chemotherapy and parenteral nutrition [34]. Studies have shown that bacteremia caused by vancomycin resistant Enterococci strains carry higher mortality rates (2.5-fold increase) as compared to bacteremia caused by vancomycin sensitive strains. In one such study the prognosis of VRE bacteremia was not much changed even with the availability of antimicrobial agents with greater potency. *E. faecalis* sigma factor Sig V that regulates gene expression in response to stress conditions has been implicated in enterococci survival and colonization in systemic infection. Absence of sig V in systemic infection in mice resulted in attenuation of bacterial translocation reducing colonization of kidney and liver. Virulence factors like Bgs A and Bgs B have also been implicated in colonization of endocarditic lesions and bacteremia. BgsA and Bgs B are now being used to treat enterococcal infections by using them as drug targets [35]. Similarly gene Asr has been implicated in *E. faecium* pathogenesis in systemic infections. Nosocomial enterococcal bacteremia have been associated with urinary catheters, intra-abdominal, burn wound, pelvic, biliary and bone sources. VRE bacteremia results in 2.5-fold increase in mortality as compared to vancomycin sensitive (VSE) bacteremia [18].

8. Infective endocarditis

Enterococci are the second most cause of infective endocarditis. Endocarditis caused by VRE faecalis causes GI or GU manipulation, damaged mitral or aortic valve infections, liver transplantation whereas VRE faecium endocarditis is associated with infection of tricuspid valve [36]. *E. faecalis* is also associated with community acquired endocarditis. Characteristic signs of infection include fever or a new murmur. Typical stigmata of endocarditis like petechiae, osler spots are rare and occur with sub-acute infections. Genitourinary infection or instrumentation often precedes the onset of enterococcal endocarditis. In published series of

enterococcal endocarditis men often outnumber women and mostly it occurred in elderly individuals. In the current therapeutic regimes, the mortality rate of enterococcal endocarditis remains around 20%.

9. Intra-abdominal and pelvic infections

VRE has been isolated from intra-abdominal and pelvic infections. The usual infections include abscesses wounds or peritonitis. Often it is a part of polymicrobial infection with Gram negative or anaerobic organisms. Usually infecting strains originate from patients intestinal flora and cause intra-abdominal infection. Enterococci are able to cause monomicrobial peritonitis infections particularly in patients undergoing chronic peritoneal dialysis or liver cirrhosis.

10. Gastrointestinal infections (GI)

GI related enterococcal infections are opportunistic infections particularly occurring during colorectal surgery and colorectal cancer. Pre-colonization with VRE in patients can result in bacteremia following antibiotic induced disruption of gut microbiota [37]. Reg IIIy, a c type lectin is secreted by intestinal epithelial and paneth cells that removes Gram positive bacteria from the gut. Antibiotic treatment causes Reg IIIy down-regulation [38]. Therapeutic strategies have been devised to prevent intestinal colonization of resistant enterococci, introducing probiotic *E. faecalis* pheromone induced killing of drug resistant *E. faecalis* reactivating Reg IIIy introducing obligate anaerobic commensal bacteria containing *Barnesiella* species which prevents *E. faecium* gut colonization and bacteremia [39]. High collagenase producing *E. faecalis* strains have been found to be associated with colorectal anastomotic leak by activating tissue matrix metalloproteinase 9 that cleaves host extracellular matrix [40]. Enterococci produce menaquinone and extracellular superoxide in intestine. This results in high oxidative stress which is linked with colorectal cancer as high genomic instability of intestinal tumor cells as around 80% of colon cancers are caused due to genetic mutations.

11. Central nervous system infections (CNS)

Although CNS infections have been reported rarely with VRE but occur in elderly patients having underlying health issues like malignancies, pulmonary and cardiac complications [41]. In them VRE *faecium* is reported at 82% and less so of VRE *faecalis.* These infections present as fever, mental disorientation, focal CNS deficits and petechial rash. CSF investigations show pleocytosis, low glucose and increased protein levels.

12. Skin and skin structure infections (SSSE)

Enterococci are part of polymicrobial infections which are found to be associated with SSSE [42]. Enterococci are frequently isolated from diabetic foot ulcers and 2–5% of patients undergoing inpatient surgery. In studies using animal models it has been seen that *E. faecalis* capsular polysaccharide in SSSI predominantly is related to the persistence of the organism. A gene cpsI encodes the carbohydrate for capsular polysaccharide.

13. Oral infections

Enterococci are inhabitants of the oral cavity and as opportunistic pathogen cause oral diseases like caries, endodontic infections, periodontitis and peri-implantitis. In endodontic infections the failure of root canal treatment by endodontic infections is now well evidenced. Enterococci have high resistance to endodontic medicaments and forms resistant biofilms. This is implicated in root canal treatment failure [43, 44]. Enterococci prevalence is also seen in gingivitis and periodontitis (3.7–35%) [45]. Oral Enterococci constitute the highest percentage of virulent genes and ability to form resistant biofilms. The oral cavity may hence be an important reservoir of virulent antibiotic resistant enterococci strains. VRE colonization occurs mainly in GI tract, skin, genitourinary tract and oral cavity. Enterococci can persist from months to years. The hands of health care workers are the most common source of transmission in nosocomial infections [46]. The need of oral care is particularly important in nosocomial settings. The spread of the nosocomial VRE occurs and when the immunity is lowered VRE multiply to cause disease. Few studies have shown that antibiotic resistant enterococci is transmitted by food [47–49] but recently Vidana et al. [50] have said there is no food related transmission of enterococci. Enterococci are now showing a high degree of resistance to tetracycline, chloramphenicol, erythromycin besides vancomycin pose a threat for spread of nosocomial infection particularly in patients of ICUs and on mechanical ventilators [51]. Vancomycin resistance is an independent predictor for the overall increase of hospital costs for the patient but also for the individual hospital [52].

14. Conclusion

VRE and have now become an important nosocomial pathogen globally. VRE causes range of infections from UTI, bacteremia, infective endocarditis, intra-abdominal and pelvic infections, central nervous system infections and even oral infections. The ability of enterococci to form recalcitrant biofilms, colonize and express virulence factors, genome plasticity, resistant to antibiotics, survival ability makes it an important nosocomial pathogen to which new therapeutic strategies have to be devised for the treatment of VRE. A periodic surveillance of VRE in hospitals is essential for limiting the spread of antibiotic resistance. Future therapy should be targeted to prevent VRE colonization of patients with immunosuppression.

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