REVIEW ARTICLE

Vancomycin resistant Enterococci: A brief review

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Abstract

Enterococci are known as opportunistic pathogens and today are accepted as leading cause of nosocomial infections. Various enterococcal species have been identified, but the major two which cause human diseases are enterococcus faecalis and enterococcus faecium. Most common and important infections caused by them are bacteraemia, endocarditis, urinary tract infections, surgical wound infections, intra-abdominal and intra-pelvic infections. Over the last two decades the emergence of vancomycin-resistant enterococci is alarming because of high mortality rate. Being resistant nosocomial infectious agents, vancomycin-resistant enterococci are a serious threat to current healthcare practices. Antibiotic resistance determinants VanA and VanB are globally reported in vancomycin-resistant enterococci clinical isolates. This paper covers a comprehensive overview of vancomycin-resistant enterococci infection epidemiology, virulence, drug resistance, its prevention and treatment.

Keywords: Enterococci spp, Drug resistance, Vancomycin.

Introduction

Enterococci (E), the gram-positive cocci, which were initially considered to be medically unimportant and believed to be harmless to humans, have now emerged as deadly nosocomial pathogens.[1] The genus exhibit remarkable array of environments and can be found in water, soil, food items like dairy and meat products, and sewage.[2] Enterococcus species are part of normal flora of almost all animals that generally colonise their gastrointestinal tracts. However, these have been isolated from sewage water and survive in other niche, like oral cavity, skin and genitourinary tract.[3]

At present, more than 30 species the genus Enterococcus have been discovered including but not limited to E. asini, E. avium, E. canis, E. casseliflavus, E. cecorum, E. columbae, E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. gilvus, E. haemoperoxidus, E. hirae, E. malodoratus, E. moraviensis, E. mundtii, E. pallens, E. phoeniculicola, E. pseudoavium, E. raffinosus, E. ratti, E. saccharolyticus, E. saccharominimus, E. solitarius, E. sulfureus, and E. villorum.[4] They are facultative anaerobes and can survive in very harsh conditions. These are catalase-negative, grow at a wide range of temperature 10-45°C and can survive at 60°C for 30 minutes. They can also grow in 40% bile salts, 6.5% sodium chloride (NaCl), in milk containing 0.1% methylene blue and at a potential of hydrogen (pH) of 9.6.[5]

The ubiquitous distribution of the genus as intestinal flora, the widespread use of broadspectrum antibiotics and invasive devices are known to be the major factors contributing to the emergence of enterococci as important pathogens. Among several factors, perhaps the most important is their extensive resistance to a wide range of antimicrobial agents. These properties allow this organism to survive and multiply with a selective advantage over other faecal flora in a hospital environment where antimicrobial agents are heavily used. This short review summarises historical background of vancomycin-resistant enterococci (VRE), its mechanisms of resistance, epidemiology, prevention and treatment.

Virulence of Enterococci

Enterococcus is a major cause of endocarditis, urinary tract infections (UTI) and bacteraemia. A review of infectious agents causing hospital acquired infections indicated Enterococci as the leading cause of nosocomial infections in United States with as high as 20%-30% and the second major cause of such infections across the world.[6] In a recent Chinese report, E. faecium presented as major pathogen (74%) followed by E. faecalis that accounted for 20% bloodstream infections with a mortality rate of 24% in total.[7] The infections caused by these organisms are tough, persistent and mostly troublesome.

The virulence of enterococci is known to be conferred by various factors including but not limited to cytolysin (CylLLLSM), Enterococcal surface protein (Esp), aggregation substance (AS), gelatinase (GelE), E. faecium cell wall adhesion factors and sex pheromones Cob and

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Ccf. The enterococcal surface protein is hypothesised to be involved in immune evasion.[8] Cytolysin has a role in progression of enterococcal infection by its haemolytic activity as well as bactericidal activity against gram-positive bacteria. AS helps in mating and conjugation at the site of infection, resulting in accumulation of bacteria at the site of infection. GelE hydrolyses haemoglobin and other peptides resulting in inflammation, and the sex pheromones can transfer plasmid carrying one or more antibiotic resistant genes.[9]

Resistant Enterococci

Antibiotic-resistant enterococci are the leading causes of hospital-acquired infections of bloodstream and urinary tract since 1980s.[10] Three main reasons behind this multidrug-resistant enterococci emergence include intrinsic resistance to antimicrobial agents like beta-lactams and aminoglycosides, and acquired resistance through mobile elements like transposon and plasmids against glycopeptides, quinolones, tetracyclines, macrolides and streptogramin or through the horizontal transfer of resistance genes.[11]

Intrinsic Resistance

The most significant resistance observed in enterococcus is resistance to aminoglycosides, beta lactamase-induced ampicillin resistance and glycopeptides. Resistance to high-level beta-lactams (minimum inhibitory concentrations [MIC] 16-64 μg/mL) is due to mutation or overproduction of penicillin binding protein 5 (PBP5). Resistance to low level aminoglycosides (MIC 62 to 500 μg/ mL) is because of slow uptake.[8]

Acquired Resistance

Mutation in the existing DNA or acquisition of foreign DNA results in acquired resistance.[8] For high-level aminoglycoside resistance (MIC >2000 μg /mL), either ribosomal mutation is acquired or aminoglycoside-modifying enzyme is acquired via plasmid.[12] Resistance to chloramphenicol is either enzymatic or plasmid-borne and resistance to high-level erythromycin is the result of transposon encoding macrolide resistance.[13] Resistance to glycopeptides can be plasmid-borne or chromosomal and is due to a cluster of genes absent in normal enterococci.[14] The MICs used for enterococcal treatment have been identified (Table).

Emergence of VRE

Since the first appearance of VRE faecalis and VRE faecium, isolated in England in 1988, the VRE has been spreading rapidly and is now detected in various hospitals all around the world.[15] Since its first use in 1958, vancomycin has been used for the treatment of gram-positive bacterial infections.[16] Enterococci become resistant to vancomycin by acquiring genes through plasmid or transposon that enable bacteria to bypass antibiotic susceptible critical steps in cell wall formation.[17]

The mode of action of vancomycin is to block the cell wall formation by targeting its building blocks.[16] It binds to the amide bond of the terminal sequences of muramylpentapeptide i.e. D-alanyl-D-alanine of the elongating peptidoglycan, thereby obstructing the polymerase extending the peptidoglycan backbone and also impedes the cross linking of the growing chain by transpeptidase.

Factors for VRE Emergence

Various factors that increase the risk of infection with VRE in a medical intensive care unit (ICU) include prolonged hospitalisation, younger age, use of ceftriaxone and vancomycin.[18] Hospital workers can also transmit VRE as it can survive on fingers for about 30 minutes even after washing hands.[8] Companion animals and pets can also be a reservoir for VRE.[19] A recent report revealed the frequency of vancomycin-resistant enterococci to be

Table: Minimum inhibitory concentrations (MIC) and zone diameter of various antibiotics are shown according to CLSI manual 2011.

S.No	Antimicrobial Agent	Disk Content	Zone Diameter S:	MIC I:	R	S:	I:	R
1	Penicillin	10 units	≥ 15	-	≤ 14	≤ 8	-	≥ 16
2	Ampicillin	10 μg	≥ 17	-	≤ 16	≤ 8	-	≥ 16
3	Vancomycin	30 μg	≥ 17	15-16	≤ 14	≤ 4	8-16	≥ 32
4	Teicoplanin	30 μg	≥ 14	11-13	≤ 10	≤ 8	16	≥ 32
5	Erythromycin	15 μg	≥ 23	14-22	≤ 13	≤ 0.5	1-4	≥ 8
6	Tetracycline	30 μg	≥ 19	15-18	≤ 14	≤ 4	8	≥ 16
7	Ciprofloxacin	5 μg	≥ 21	16-20	≤ 15	≤ 1	2	≥ 4
8	Levofloxacin	5 μg	≥ 17	14-16	≤ 13	≤ 2	4	≥ 8
9	Rifampin	5 μg	≥ 20	17-19	≤ 16	≤ 1	2	≥ 4
10	Chloramphenicol	30 μg	≥ 18	13-17	≤ 12	≤ 8	16	≥ 3 2
11	Linezolid	30 μg	≥ 23	21-22	≤ 20	≤ 2	4	≥ 8

11.3% from a tertiary care hospital of Pakistan.[20]

Animal husbandry can serve as a source of VRE due to use of antibiotics for prophylaxis or growth promotion. Avoparcin, a glycopeptides analogue of vancomycin, is related to the high prevalence of VRE in animals and a source of transmitting VRE to healthy people having no hospital exposure.[21] In 1933, the first report about VRE occurrence in a non-human source was published. Excessive amount of avoparcin along with other antimicrobials was used as feed additive particularly in Europe. In animals having avoparcin as food additive, VRE readily colonise the intestinal tract which ultimately results in colonisation of human intestinal tract.[11] The avoparcin usage was banned by Council of Agriculture in various countries as a precautionary measure regarding issues related to human health. These countries include Denmark and Norway (1995), Germany (1996), the rest of Europe and Korea (1997), Taiwan and New Zealand (2000).[22] In the United States, avoparcin was never approved for use in animal feed and presence of VRE in US is attributed to clinical settings.[11]

Lopez et al. in 2009 reported VRE in 4% of food samples of animal origin even after 10 years of the ban of avoparcin.[23] The continual presence of VRE in these farms and in food samples after avoparcin ban suggests a linkage between resistance determinants to antibiotics such as macrolide and vancomycin resistance genes. The macrolide resistance gene ermB is supposed to be co-transferred to vancomycin resistance gene (VanA) as both genes are present on the same conjugative plasmid.[23] The persistence of VRE in farms can also be attributed to the ability of VRE to remain viable for a long period of time.

Morbidity and Mortality Rate of VRE faecium

Saka et al. in 2008[24] hypothesised that VRE bacteraemia was particularly due to "exogenous" sources such as allogenic bone marrow transplant, exposure to chemotherapeutic agent, hypo-albuminaemia and urinary catheter. While for vancomycin-sensitive enterococci (VSE) bacteraemia "endogenous" sources were the major cause of pathogenecity like age, prior gastrointestinal disease, and abdominal surgery. VRE colonisation has been shown to be present universally in patients who are developing bacteraemia. Vancomycin resistance is associated with the high mortality rate by VRE. Hence, clinical settings should be improved and infection control practices should be adapted in addition to control of excessive use of antibiotics to reduce the occurrence of VRE bacteraemia.

Resistance Markers of Vancomycin

Resistance markers of vancomycin have been classified in two ways; phenotypically and genotypically.

Phenotypic Classification

Until now, nine types of resistance genes have been identified for the genus enterococcus: VanA, VanB, VanC, VanD, VanE, VanG, VanL, VanM and VanN. These genes confer high to moderate level resistance to vancomycin and teicoplanin.[25] Among nine types of resistance genes, VanA and VanB phenotypes are the most prevalent in VRE, causing infections in humans.[26] The VanA phenotype confers resistance to both vancomycin (MICs > 64 μg/mL) and teicoplanin (MICs >16 μg/mL) and is dominant among all genotypes.[14] VanB phenotype is inducibly resistant to modest levels of vancomycin (MICs 32 to 64 μg/mL) to high levels of vancomycin (4 to > 1,000 μg/mL) but show susceptibilty towards teicoplanin.[8]

Genotypic Classification

In enterococci, under normal conditions, peptidoglycan synthesis is carried out by a ligase enzyme that joins two molecules of D-alanine to form D-Ala-D-Ala. The UDP-N-acetylmuramylpentapeptide forms by addition of D-Ala-D-Ala to UDP-N-acetylmuramyltripeptide. Through transglycosylation, the pentapeptide is incorporated into peptidoglycan and via transpeptidation crossbridges form to strengthen the peptidoglycan layer.[8]

The VanA gene cluster is plasmid-borne and encoded by a 10,851-bp trasposon identified as Tn1549 that makes the gene transferrable.[23] The VanB gene cluster is present on chromosome and is encoded by a mobile element of approximately 60 kb named as Tn1547. The VanB gene cluster has also been found on 50 to 60 kb plasmids.[27]

VanA Glycopeptide Resistance

The VanA gene is not responsible for conferring resistance alone but there are other genes which regulate and express the resistance. These genes are: VanR, VanS, VanH, VanX, VanY and VanZ all of which are located on Tn1549. These genes express to synthesise the abnormal precursor of peptidoglycan to which vancomycin binds with very low affinity.

The VanA protein 38-40 kD acts as a ligase which has specificity for D-Ala-D-Lac, the altered molecule. VanH protein acts as a D-hydroxy dehydrogenase and creates a pool of altered substrate i.e. D-lactate. VanX protein has D, D-dipeptidase activity for D-Ala-D-Ala to reduce its pool for normal ligase, thereby normal pentapeptide competitive synthesis is minimised. The VanR and VanS proteins have regulatory function. The VanHAX gene cluster is regulated by these regulatory proteins, where

VanS acts as a sensor that detects vancomycin presence and signals VanR to activate the synthesis of VanHAX genes. The role of VanY and VanZ in vancomycin resistance is not well-defined.[8] The VanZ confers low level teicoplanin resistance through an unknown mechanism.[13]

VanB Glycopeptide Resistance

The resistance level (MICs, 4->256 mg/mL) in vanB is lower than VanA and is attributed to VanB ligase enzyme that converts D-Ala-D-Ala to D-Ala-D-Lac, perhaps due to less substitution.[28] The genes of VanB gene cluster are designated as VanHB, VanXB, VanYB, VanRB, and VanSB. However, there is no gene homologous to VanZ but there is a gene of unknown function, VanW. The VanB isolates appear to be susceptible to teicoplanin, as VanRB-VanSB system is induced by vancomycin but not by teicoplanin.[29] Other differences are also present in VanA- and VanB-type resistances. More widely distributed and predominant type of resistance reported from Europe is VanA whereas VanB strains are fairly common in the US with some hospitals reporting VanB exclusively but VanA isolates are still dominant.[8]

Vancomycin Dependence Paradox

A phenomenon that has gained attention of scientists is the dependence of VanA type VRE on vancomycin. Vancomycin-dependent enterococci (VDE) require vancomycin for their growth and are resistant to it as well. Vancomycin acts as an inducer thereby inducing VanA and VanH to make D-Ala-D-Lac. It is probably because in the presence of vancomycin, enterococci do not need the normal D-Ala- D-Ala for their cell wall synthesis, indeed it is destroyed by VanX and D-Ala-D-Lac is used as a precursor for cell wall synthesis. Removal of vancomycin stops the synthesis of D-Ala-D-Lac and bacteria cannot survive in the absence of either of the precursor of cell wall.[8]

Treatment

If the enterococci does not exhibit high-level resistance to the beta-lactams, amino glycosides or glycopeptide then the combination of a beta-lactam or glycopeptide and aminoglycoside can have bactericidal effect.[23] A cell wall active agent (glycopeptides or beta-lactam) blocks the synthesis of peptidoglycan thereby permitting the entrance of amino glycoside and hence this synergy can have bactericidal effect.[12] Treatment options for VRE include tigecycline, linezolid, daptomycin, quinipristin-dalfopristin, platensimycin, nitrofurantoin and fosfomycin with some reports of resistance as well.[30] Modifications in the structure of vancomycin have also shown great promise for treating VRE.[16]

Prevention and Control

The prevention and control strategy for VRE should include limiting excessive use of vancomycin and cephalosporins, avoiding unnecessary hospitalisation, hospital staff training for prompt screening and reporting of VRE, and employing non-touch environmental cleaning to prevent person-to-person transmission.[30]

Recommendations

VRE have widespread distribution patterns in various environmental and clinical niches. The extensive use of invasive devices, broadspectrum antibiotics and increased hospital stay are major factors contributing to the emergence of VRE. Multipronged strategy focussing on limiting the unnecessary use of broadspectrum antibiotics like vancomycin and cephalosporins, and decreasing the duration of hospitalisation should be implemented to prevent the emergence and spread of VRE. Standard protocols be employed for controlling infections spread in hospitals, including, but not limited to, non-touch cleaning, immediate identification and reporting of VRE and hospital staff hygiene trainings. Multi-centre large studies are warranted for creating indigenous VRE database for Pakistan.

Conclusion

Presence of enterococci as intestinal flora, ever increasing use of medical devises, prolonged hospitalisation and, most importantly, irrational and improper antibiotics therapy, have resulted in the emergence of VRE. Enterococcal arsenal for combating antibiotics includes enzymes that turn antibiotics undamaging to them. This serious issue of increasing VRE prevalence has been reported from across the world. VRE bacteraemia claims more mortality and morbidity all over the world. Developing countries with less stringent control over availability and irrational prescribing of broadspectrum antibiotics are more vulnerable to these serious health crises. The previously thought looming antibiotic void and so-called post antibiotic era seems to have already approached, albeit less reported in the developing world.

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