

Healthcare associated vancomycin- resistant enterococci

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ABSTRACT: Vancomycin resistant enterococci (VRE) were first detected in the 1980s, since then VRE infections are reported worldwide. VRE can be isolated from healthy people, hospital environment and from farmed chicken. VRE infections are more severe, increased mortality and economic burden. Healthcare associated infections are mainly due to antibiotic therapy, patient characteristics, colonization pressure and exposure to contaminated environment. VRE are resistant to most commonly used antibiotics, with the ability to acquire resistance to other available antimicrobials and to transfer antibiotic resistance to other gut flora. CDC Hospital Infection Control Practices Advisory Committee (HICPAC) recommendations are useful to minimize transmission of VRE with multidisciplinary approach, and restricted cephalosporin usage has been associated with decreased VRE transmission in hospitals.

KEY WORDS: Vancomycin, *Enterococcus faecium*. Resistance mechanisms, VRE control

I. INTRODUCTION

Vancomycin –resistant *Enterococcus faecalis* and *Enterococcus faecium* clinical isolates were first documents in Europe in the late 1980s [1,2]. Since then, VRE have been associated with outbreaks of hospital acquired (nosocomial) infections around the world. In the United States from 2006 to 2007, 4% of vancomycin-resistant *E. faecium* was healthcare- infections [3]. VRE can be carried by healthy people who come into contact with the bacteria. The most likely place where such contact can occur is in the hospital although it is thought that a significant percentage of intensively farmed chicken also carry VRE [4,5]. Compared with vancomycin susceptible enterococci (VSE), VRE infections cause more severe disease, increased mortality and have significant additional economic burden. Antibiotic treatment options for VRE are limited and pan-resistance can occur [6]. Enterococci with acquired transferable high level resistance to vancomycin were first detected in feces from two leukemia patients from Europe in 1986 [7]. Hospital outbreaks were increasingly described over the next decade across Europe and the USA. Internationally, the important resistance genotypes of VRE have been either *vanA* or *vanB* operons usually carried by *Enterococcus faecium* in a transposon located within a large transferable plasmid. There is evidence of global spread of clonal complex of hospital-associated ampicillin resistant *E. faecium* (CC17) that includes both VSE and VRE (either *vanA* or *vanB*) and has a number of putative virulence factors for hospital adaptation and spread [8,9]. Researchers in Japan reported patients infected or colonized with *Enterococcus faecium vanB* [10]. In Australia, *vanB-E. faecium* has predominated since late 1990s. A 2010 Australian VRE survey indicated increasing prevalence of VRE *vanBE. faecium* from the CC17 in a number of States [11]. Risk factors for healthcare-associated colonization and or infections with VRE have been identified, they include: (a) previous antibiotic exposure, (b) patient characteristics, (c) colonization pressure, (d) exposure to contaminated surfaces [12]. Control measures of VRE include the enhanced standard precautions (horizontal measures) in healthcare that reduce infections caused by all organisms, not just VRE. Effective antimicrobial stewardship programs are paramount and should target reduction in the use of extended-spectrum cephalosporins, carbapenems and fluoroquinolones [13]. This paper reviews mechanisms of resistance, epidemiology, and control of VRE.

II. MECHANISMS OF RESISTANCE

Among enterococci seven types of glycopeptide resistance have been described (VanA, VanB, VanC, VanD, VanE, VanG, and Van I) which are based on their specific ligase genes (e.g., *vanA*, *vanB*, etc). Glycopeptide resistance in enterococci is classified as either intrinsic (as a species characteristic) or acquired. The former is a characteristic of the motile species *Enterococcus gallinarum* and *Enterococcus casseliflavus*, these strains carry *vanC-1* and *vanC-2/vanC-3* genes. Acquired resistance is found most often in *E. faecium* followed by *E. faecalis*, and is much less common in other enterococcal strains [14 15].

Resistance to β -lactam

Complete or relative resistance to β -lactams is a characteristic feature of the genus *Enterococcus*. *E. faecalis* is typically 10 to 100 times less susceptible to penicillin than other streptococci, while *E. faecium* is at least 4 to 16 times less susceptible than *E. faecalis* [16]. While most isolates of *E. faecalis* are inhibited by concentrations of penicillin or ampicillin (1 to 8 $\mu\text{g/ml}$) easily achievable in humans, isolates of *E. faecium* usually require an average of 16 to 64 $\mu\text{g/ml}$ to inhibit growth, although some isolates are more resistant [17]. An additional problem with enterococci is that they are typically tolerant to β -lactams (i.e. MBC/MIC of >32). The major mechanism underlying this resistance has the production of low-affinity PBP [18]. Fontana et al. showed that loss of the ability of a strain of *E. faecium* to produce PBP5 caused this highly penicillin-resistant strain to become hyper susceptible to penicillin [19]. β -lactamase production is inducible, β -lactamase production in enterococci is constitutive low level, and inoculum dependent [20].

Resistance to Aminoglycoside

Early researchers demonstrated that two types of streptomycin resistance occur in enterococci: (i) moderate level resistance (MIC, 62 to 500 $\mu\text{g/ml}$), because of low permeability, which can be overcome with penicillin (which increases the cellular uptake of the aminoglycoside); and (ii) high level resistance (MIC, $\geq 2,000$ $\mu\text{g/ml}$), which is either ribosomally mediated or due to the production of aminoglycoside-inactivating enzymes [21]. Since enterococcal resistance to gentamicin and streptomycin occur by different mechanisms, it is important to test susceptibilities to both agents. Gentamicin resistance is predominantly the result of the presence of the inactivating enzyme 2''-phosphotransferase-6'-acetyltransferase conferring resistance to gentamicin, tobramycin, netilmicin, amikacin and kanamycin. Hence gentamicin resistance is a good predictor of resistance to other aminoglycosides except streptomycin is encountered mainly in enterococcal strains that produce streptomycin adenyltransferase; these strains remain susceptible to gentamicin [20, rept].

Resistance to Vancomycin

There are five recognized phenotypes of vancomycin resistance, VanA, VanB, VanC, VanD, and VanE [22]. Two of these (VanA and VanB) are mediated by newly acquired gene clusters not previously found in enterococci. VanA and VanB resistance phenotypes were mainly described primarily in *E. faecalis* and *E. faecium*. VanA-resistance strains possess inducible, high-level resistance to vancomycin (MICs ≥ 64 $\mu\text{g/ml}$) and teicoplanin (MICs ≥ 16 $\mu\text{g/ml}$) [22]. Resistance can be induced by glycopeptides (vancomycin, teicoplanin, avopercin, and ristocetin) and nonglycopeptide agents such as bacitracin, polymixin B, and robinidazole, a drug used to treat coccidial infections in poultry [23]. The details of vancomycin resistance have been best documented with the *vanA* gene cluster found on the transposon, or "jumping" genetic element, *Tn1546* [22].

Resistance mechanisms and genetic classification

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-Ala, which is then added to UDP-N-acetylmuramyl-tripeptide to form the UDP-N-acetylmuramyl-pentapeptide. The UDP-N-acetylmuramyl-pentapeptide, when incorporated into the nascent peptidoglycan (transcosylation), permits the formation of cross bridges (transpeptidation) and contributes to the strength of the peptidoglycan layer [24]. 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 [25].

VanA glycopeptide resistance

The *vanA* gene and other genes involved in the regulation and expression of vancomycin resistance (*vanR*, *vanH*, *vanX*, and *vanZ*) are located on a 10,581-bp transposon (*Tn1546*) of *E. faecium*, which often resides on a plasmid [26]. Expression of these genes results in the synthesis of abnormal peptidoglycan precursors terminating in D-Ala-D-lactate instead of D-Ala-D-Ala. Vancomycin binds to D-Ala-D-Lac with marked lower affinity than it does to the normal dipeptide product [27]. The core protein functions favoring synthesis of pentapeptide terminating in D-Ala-D-Lac are as follows. (a) VanA protein is a ligase of altered substrate specificity which produces D-Ala-D-Lac in preference to D-Ala-D-Ala [28]. (b) VanH protein is a D-hydroxy acid dehydrogenase which creates a pool of D-lactate for use in the above reaction [29]. (c) 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 enterococcal ligase, thereby minimizing the competing synthesis of normal pentapeptide [25, rept]. VanA alone cannot confer resistance to vancomycin, probably because D-hydroxy acids such as D-Lac are neither natural products present in the environment of enterococci nor produced by enterococci [30].

VanR and VanS proteins constitute a two-component regulatory system that regulates the transcription of *vanHAX* gene cluster [31]. VanS apparently functions as a sensor to detect the presence of vancomycin [22] or, more likely, some early effect of vancomycin on cell wall synthesis [32]. VanS then signals VanR the response regulator, which results in activation, or turning on of the synthesis of some other proteins (VanH, VanA, and VanX) involved in resistance. In VanA phenotype strains, either vancomycin or teicoplanin can induce the transcription, but the precise signals are still unknown [33]. *vanY* and *vanZ* may contribute to but are not essential for 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 vancomycin, though mechanisms that have not yet been elucidated. It is not essential to the expression of VanA phenotype [34]. Mapping of the *vanA* gene cluster from several U.S. isolates revealed some heterogeneity in organization [35,36]. 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 additional insertion like elements [35,36].

Cross-linking of the precursors to the growing peptidoglycan is processed in bacteria by the PBPs, with PBP₅ being used in enterococci [37]. 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 so far not known to play a role in cell wall synthesis, are probably required for processing the altered precursors [38]. These high-molecular weight PBPs display a higher affinity for β -lactams. Since VanA resistance is inducible, a shift in the PBPs occurs only in the presence of vancomycin and results in β -lactam hypersusceptibility. This effect explains the synergy displayed by the combination of the two classes of drugs against vancomycin-resistant strains [39].

VanB glycopeptide resistance in enterococci is mediated by an abnormal ligase (VanB) that is structurally related to VanA (76% amino acid identity). VanB protein also favors the production of the pentadepsipeptide terminating in D-Ala-D-Lac [40]. Genes analogous to their class A resistance counterparts are designated *vanH_B*, *vanX_B*, *vanY_B*, *vanR_B*, and *vanS_B* [41]. Levels of D,D-dipeptidase activity (*vanXB*) correlates with levels of vancomycin resistance [42]. There is high degree of sequence identity (approximately 70%) between *vanHAX* and *vanHBBXB* but considerably less homology (25 to 35% sequence homology) between RS and Y proteins of VanA and VanBVRE [42]. There is no gene counterpart of *vanZ* in these organisms. *vanY_B* is not found in all strains, and its position in the gene clusters differs from that of *vanY* in Tn1546 [43]. Recent reports have shown DNA sequence heterogeneity, suggesting three subtypes of *vanB* ligase gene: *vanB-1*, *vanB-2*, and *vanB3* [44]. The regulatory system in class B strains appears insensitive to induction by teicoplanin [42, rept]. Teicoplanin induces the synthesis of VanA-related proteins but does not induce the production of VanB-related proteins. On the other hand, vancomycin induces the synthesis of the resistance proteins of both systems, and in fact, if a teicoplanin susceptible enterococcus with *vanB* gene cluster is preexposed to vancomycin, the strain then tests teicoplanin 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 [17]. 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 inducer. [39].

VanC glycopeptide resistance. Enterococcus strains i.e. *E.gallinarum*, *E.casseliflavus* and *E.flavescence* exhibit low level resistance to vancomycin. 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.flavescence* have been reported, there is some disagreement about whether *E.flavescence* is a legitimate enterococcal species [45]. VanC ligase of *E.gallinarum* favors the production of a pentapeptide terminating in D-Ala-D-Ser. Substitution of D-Ser for D-Ala is presumed to weaken the binding of vancomycin to the novel pentapeptide. Insertional inactivation of *vanC-1* unmasks the concomitant production of the D-Ala-D-Ala pentapeptide in *E.gallinarum* [46]. D,D-Dipeptidase and D,D-carboxypeptidase activities analogous to those 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 [47].

VanD glycopeptide resistance. A novel vancomycin resistance gene designated *vanD* was first described in a New York Hospital in 1991 [48]. The strain carrying this resistance trait was an *E.faceium* strain that was inhibited by vancomycin at 64 μ g/ml and teicoplanin at 4 μ g/ml. Partial sequencing of the ligase gene showed that it was distinct from but similar to the *vanA* and *vanB* genes. Recently three clinical isolates of vancomycin resistant *E.faceium* carrying the *VanD* resistance trait were found in Boston [49], 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 [39].

VanEglycopeptideresistance. The vane vancomycin resistance gene has recently been described in *E. faecium* BM4405, which is resistant to low levels of vancomycin (MIC, 16 µg/ml) and susceptible to teicoplanin (MIC 0.5 µg/ml). [50]. This new genotype has similarities to the intrinsic VanC type of resistance. The deduced amino acid sequence has a greater identity to VanC (55%) than to VanA (45%) or VanD (44%). [50]. *Lactobacillus casei*, *Pedococcus spentosacus* and *Leuconostoc mesenteroides* are naturally resistance to glycopeptides [51].

Vancomycin-dependent enterococci (VDE). An interesting phenomenon that has developed in some strains of VanA- and VanB type VRE is that of vancomycin dependence [52]. These enterococci not just resistant to vancomycin but now require it for growth. Vancomycin dependent enterococci have been recovered from apparently culture- negative clinical samples by plating them onto vancomycin-containing agar such as that used for *Campylobacter* or gonococci. A likely explanation for the phenomenon of vancomycin dependence is that these enterococci turn off their normal production of D-Ala-D-Ala and grow only if a substitute dipeptide-like structure is made [17].

III. CONTROL OF VRE IN HELTHCARE SETTING

Epidemiology and beginning of VRE strains spread in Europe and the United States have notable differences. In Europe, glycopeptide avoparcin was frequently fed to animals as a growth enhancer, apparently selecting for the VRE commonly found in the intestinal flora of animals. The contamination of food from animals, such as poultry products, presumably led to the VRE colonization seen in a number of healthy individuals from European countries. In the United States, on the other hand, glycopeptides have never been approved for animal feed use and VRE carriage was largely undetected. (except the endogenously resistant species *E. gallinarum* and *E. casseliflavus*) outside health care setting, at least early on. The wide use of vancomycin in the hospital setting is likely one of the culprits for rapid selection and proliferation of VRE within this environment. The proportion of VRE among enterococcal isolates from nosocomial infections has continued to rise during recent years, although with a lower rate of increase, as reported by the Centers for Disease Control and prevention (CDC) [53].

VRE transmission

VRE transmission by health care workers whose hands become transiently contaminated with the organism while caring for infected patients probably the most common mode of nosocomial transmission. This concept is supported by the recovery of VRE and other resistant enterococci from cultures of specimens from hands of health care workers [54]. VRE transmission may also occur by way of contaminated medical equipment, although this is probably much less important than transmission by the hands of personnel. Electronic thermometers contaminated with outbreak strain were epidemiologically implicated in an outbreak [55]. The spread of VRE via bedpan washer machines has been reported (PR. Chadwick *et al*, Letter, *Lancet* 344:685, 1994). Nosocomial transmission of VRE has been attributed to the use of fluidized microsphere bed from which multiply resistant strains were recovered despite repeated attempts at decontamination by the manufacturers [56]. Enterococci have been recovered from 7 to 30% of environmental cultures during several outbreaks [57]. Since enterococci remain viable for several days to weeks on dry surfaces, it seems plausible that contaminated surfaces may act as a source from which personnel may contaminate their hands or clothing [57].

Disposable cover gowns worn by personnel who care for VRE patients have also been shown to be contaminated with patient's organism [58]. Presumably, the clothing of the personnel who do not wear cover gowns may also become contaminated with VRE. However, at present there is no conclusive proof that VRE are spread by contaminated clothing. There is no proof that enterococci, including VRE, are spread by the airborne route. Recovery of VRE from animal sources in parts of Europe suggests that food borne transmission may occur in certain geographic areas [59].

Prevention and control of VRE

The epidemiology of VRE has not been elucidated completely; however, certain patients populations are at increased risk for VRE infection or colonization. These include critically ill patients or those with severe underlying disease or immunosuppression, such as ICU patients or patients on oncology or transplantation wards, those who have had an intra-abdominal or cardiothoracic surgical procedures, those with an indwelling urinary or central venous catheter, and those who have had a prolonged hospital stay or received multiple antimicrobial agents [57]. CDC, hospital Infection Control Practices Advisory Committee (HICPAC) published recommendations in February 1995 [60]. These recommendations mainly focused on (i) prudent use of vancomycin, (ii) education of hospital staff, (iii) effective use of microbiology laboratory, and (iv) implementation of infection control measures. To minimize nosocomial transmission of VRE, hospitals must use multidisciplinary approach that requires participation by a variety of departments and personnel [60].

Restricted usage of cephalosporin has been associated with decreased VRE infection and transmission in hospitals [61]. Antimicrobial stewardship (AMS) is fundamental in the control of major hospital pathogens. In particular, restriction of the use of extended- spectrum cephalosporins, quinolones is of proven worth for VRE, MRSA, MRGN and *C.difficile* [62].

Control measures recommended by HICPAC to prevent patient to –patient transmission of VRE include: (a) Place VRE-infected or-colonized patients in single rooms or in same room as other patients with VRE [57].(b)Wear nonsterile gloves when entering the room of a VRE –infected or-colonized patient. During the course of caring patient, a change of gloves may be necessary after contact with material that may contain high concentrations of VRE[57].(c)Wear a clean nonsterile gown when entering the room of a VRE-infected or-colonized patient if substantial contact with the patient or environmental surfaces in the patient’s room is anticipated od if patient is incontinent or has diarrhea, an ileostomy,or wound drainage and contained by dressing [57] (d) Remove gloves and gowns before leaving the patient’s room and wash hands immediately with an antiseptic soap or use waterless agent [63].Hands can be contaminated via glove leaks or during glove removal, and bland soap is relatively ineffective in removing VRE from the hands[63].(e)Ensure that after glove and gown removal and hand washing clothing and hands do not contact environmental surfaces potentially contaminated with VRE(e.g., door knob or curtain) in the patient’s room [57].Although it is not recommended by HICPAC ,some hospitals require that gowns and gloves be worn routinely by all personnel entering VRE patient’s room [57].Anecdotal experience in an ICU in which outbreaks caused by a single clone of VRE occurred revealed that routinely wearing gowns and gloves before entering the rooms of patients, combined with other infection control measures, was effective in terminating the outbreaks [57].Handwerker *et. al.* also reported that the use of gowns in addition to other infection control procedures terminated outbreaks [64].The HICPAC has some additional recommendations for hospitals with endemic VRE or continued VRE transmission despite the implementation measures [60].In 2005,*Lactobacillusrhammosus* GG(LGG),a strain of *L.rhammosus*, was used successfully for first time to treat gastrointestinal carriage of VRE in renal patients [65].

CONCLUSION

Transmission control of vancomycin resistant enterococci (VRE) Include CDC Hospital Infection Control Practices Advisory Committee (HICPAC) recommendations and restricted usage of vancomycin and cephalosporin in the hospital.

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