



Symposium 2.1

Resistance Mechanisms of VRE

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The first isolates of high-level vancomycin-resistant enterococci (VRE) were reported by investigators in the United Kingdom two decades ago. Now, VRE are a worldwide challenge.

Six glycopeptide resistance types, VanA, VanB, VanC, VanD, VanE and VanG, have been described in enterococci; they can usually be distinguished on the basis of the level, inducibility, and transferability of resistance to vancomycin and teicoplanin, although they are most specifically distinguished by their associated genes (table 1). The first two types are the most clinically relevant.

The genes associated with high level vancomycin resistance in enterococci, *vanA*, *vanB*, and *vanD*, encode a ligase responsible for the synthesis of the depsipeptide D-alanyl-D-lactate which is incorporated into a pentapeptide peptidoglycan cell wall precursor (which terminates in D-alanyl-D-lactate) to which vancomycin binds poorly (figure 1). In contrast, in vancomycin-susceptible cells, vancomycin complexes with the D-alanyl-D-alanine termini of normal pentapeptide peptidoglycan cell wall precursor thereby inhibiting cell wall synthesis (figure 1).

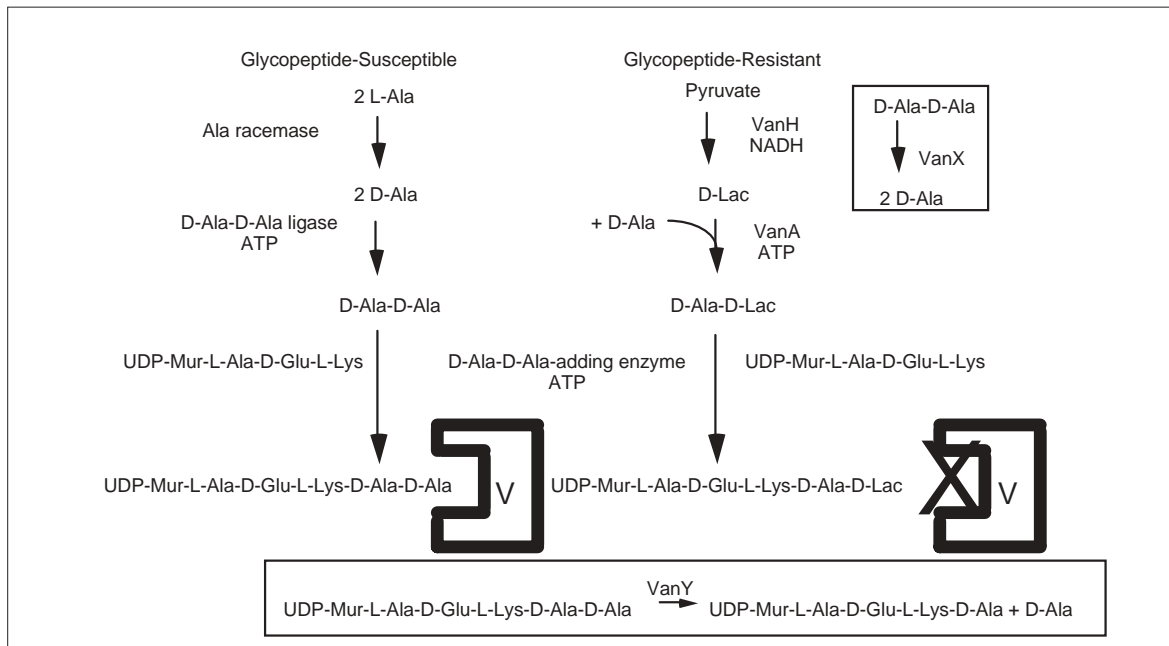
VanA-type phenotypic glycopeptide resistance has traditionally been characterized by acquired inducible resistance to both vancomycin and teicoplanin, although there can be variability in the phenotype (9, 12, 14, 20, 26). VanA resistance is mediated by Tn 1546 or closely related elements. Tn 1546 consists of 10,851 base pairs and encodes nine polypeptides that can be assigned to different functional groups: Transposition functions, regulation of glycopeptide resistance genes (VanR and VanS), synthesis of depsipeptide D-alanyl-D-lactate which when incorporated into the pentapeptide peptidoglycan precursor form a pentapeptide precursor to which neither vancomycin nor teicoplanin will bind (VanH and VanA), and hydrolysis of precursors of normal peptidoglycan (VanX and VanY) (figure 2) (1). VanY and VanZ are accessory peptides not required for resistance. Genetic heterogeneity has been described in the *vanA* gene clusters of VRE, and the *vanA* gene cluster has been found on the chromosome as well as plasmids.

Table 1. Resistance to glycopeptides in enterococci

Phenotype	Genotype MIC (µg/ml)	Vancomycin MIC (µg/ml)	Teicoplanin	Expression	Transfer	Species
VanA	<i>vanA</i>	64->1000	16-512	Inducible	+	<i>E. faecium</i> <i>E. faecalis</i> <i>E. avium</i> <i>E. gallinarum</i> <i>E. durans</i> <i>E. mundtii</i> <i>E. casseliflavus</i> <i>E. raffinosus</i> <i>E. hirae</i>
VanB	<i>vanB</i>	4-1000	0.25-2	Inducible	+	<i>E. faecium</i> <i>E. faecalis</i> <i>E. gallinarum</i> <i>E. durans</i>
VanC	<i>vanC-1</i>	2-32	0.12-2	Constitutive Inducible	-	<i>E. gallinarum</i>
VanC	<i>vanC-2</i>	2-32	0.12-2	Constitutive	-	<i>E. casseliflavus</i>
VanC	<i>vanC-3</i>	2-32	0.12-2	Constitutive	-	<i>E. flavescens</i>
VanD	<i>vanD</i>	16-256	2-64	Constitutive	-	<i>E. faecium</i> <i>E. faecalis</i> <i>E. raffinosus</i>
VanE	<i>vanE</i>	16	0.5	Inducible	-	<i>E. faecalis</i>
VanG	<i>vanG</i>	16	0.5	Inducible	-	<i>E. faecalis</i>

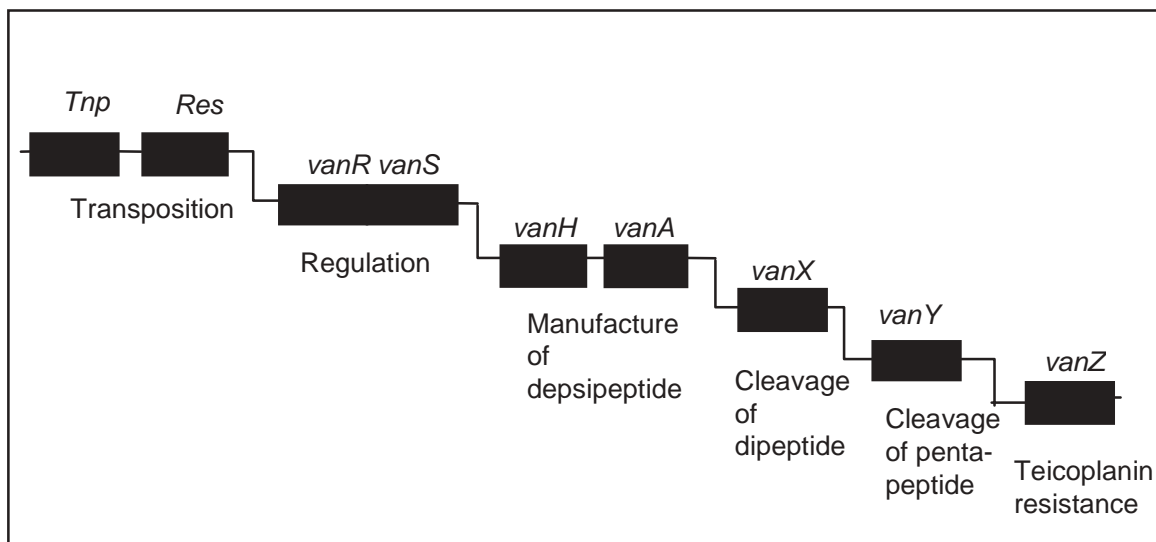
VanB-type glycopeptide resistance is classically characterized by acquired inducible resistance to various concentrations of vancomycin but not to teicoplanin. The *vanB* gene cluster, as described in *Enterococcus faecalis* V583, has homology to the *vanA* gene cluster; it consists of genes encoding polypeptides assigned to regulation of glycopeptide resistance genes (*vanRB* and *vanSB*), synthesis of the depsipeptide D-alanyl-D-lactate (*vanHB* and *vanB*), and hydrolysis of precursors of normal peptidoglycan (*vanXB* and *vanYB*). Several groups, including ours, have identified sequence variability in *vanB* amongst different enterococcal isolates (21).

Figure 1. Mechanism of action of vancomycin and mechanism of vancomycin resistance in enterococci with *vanA*-associated vancomycin resistance (V, vancomycin).



VanC-type glycopeptide resistance is characterized by low-level vancomycin resistance but teicoplanin susceptibility and is an intrinsic property of *Enterococcus gallinarum*, and *Enterococcus casseliflavus/flavescens*. Pentapeptide peptidoglycan precursors in isolates with VanC vancomycin resistance terminate in D-alanyl-D-serine rather than in D-alanyl-D-alanine.

Figure 2. The *vanA* operon in Tn 1546



High-level vancomycin resistance has been described in *Staphylococcus aureus*, as a result of transfer of vancomycin resistance-associated genes from *Enterococcus faecalis* (29). *vanA* has been found in vancomycin-resistant clinical isolates of *Oerskovia turbata*, *Arcanobacterium haemolyticum*, and *Bacillus circulans* (5, 22), and *vanB* in glycopeptide-resistant isolates of *Streptococcus bovis* and *Streptococcus gallolyticus* and anaerobic bacteria present in human feces (4, 23, 27).

Gene clusters similar to *vanHAX* and *vanH_BBX_B* have been identified in several soil dwelling glycopeptide-producing members of the bacterial order *Actinomycetales* (18). A complete vancomycin resistance system has also been identified in the glycopeptide producing (and glycopeptide resistant) actinomycete, *Streptomyces coelicolor* (11). We have shown that *Paenibacillus popilliae* contains *vanF* encoding a putative D-alanine:D-lactate ligase, VanF, as part of the *vanR_FS_FY_FZ_FH_FFX_F* cluster similar in structure to the enterococcal *vanA* and *vanB* clusters (25). We have also found a short sequence of DNA with homology to the enterococcal Tn 1546 transposase gene within the glycopeptide resistance cluster of several strains of *P. popilliae* possibly representing a "footprint" of a Tn 1546-like element that was necessary for an intermediate combinational step in the molecular evolution of the *P. popilliae* glycopeptide resistance cluster (6).

Over the past few years, linezolid resistance has emerged in vancomycin-resistant enterococci (3, 7, 10, 13, 16, 24, 28). Herrera et al. reported nosocomial transmission of linezolid-resistant vancomycin-resistant enterococci (10). In almost all clinical studies, linezolid resistance has been associated with a G2576T mutation in the 23S rRNA gene (2, 3, 10, 13, 24, 28). One study, however, reported a U2357A mutation in the 23S rRNA gene (in the absence of the G2576T mutation) associated with linezolid resistance in *E. faecium* (16). Bonora et al. reported several linezolid-resistant vancomycin-resistant enterococcal isolates from intensive care unit patients that belonged to different clones (3). Bae et al reported the first clinical isolate of linezolid- and vancomycin-resistant *Enterococcus faecium* in Korea; the minimum inhibitory concentration of linezolid was proportional to the number of copies of the G2576U mutation (2). Daptomycin resistant in vancomycin-resistant enterococci has also being reported (8, 15, 17, 19).

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