Vancomycin Resistance in Gram-Positive Cocci

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The first vancomycin-resistant clinical isolates of *Enterococcus* species were reported in Europe in 1988. Similar strains were later detected in hospitals on the East Coast of the United States. Since then, vancomycin-resistant enterococci have spread with unexpected rapidity and are now encountered in hospitals in most countries. This article reviews the mode of action and the mechanism of bacterial resistance to glycopeptides, as exemplified by the VanA type, which is mediated by transposon Tn*1546* and is widely spread in enterococci. The diversity, regulation, evolution, and recent dissemination of methicillin-resistant *Staphylococcus aureus* are then discussed.

The first vancomycin-resistant clinical isolates of *Enterococcus* species were reported in Europe in 1988 [1, 2]. Similar strains were later detected in hospitals on the East Coast of the United States [3]. Since then, vancomycin-resistant enterococci have spread with unexpected rapidity and are now encountered in hospitals in most countries [4].

MODE OF ACTION OF VANCOMYCIN

The synthesis of peptidoglycan in the production of bacterial cell walls requires several steps. In the cytoplasm, a racemase converts l-alanine to d-alanine (d-Ala), and then 2 molecules of d-Ala are joined by a ligase, creating the dipeptide d-Ala-d-Ala, which is then added to uracil diphosphate–*N*-acetylmuramyl-tripeptide to form uracil diphosphate–*N*-acetylmuramyl-pentapeptide. Uracil diphosphate–*N*-acetylmuramyl-pentapeptide is bound to the undecaprenol lipid carrier, which, after the addition of GlcNAc from uracil diphosphate–GlcNAc, allows translocation of the precursors to the outer surface of the cytoplasmic membrane. *N*-acetylmuramyl-pentapeptide is then incorporated into nascent peptidoglycan by transglycosylation and allows the formation of cross-bridges by transpeptidation [5].

Vancomycin binds with high affinity to the d-Ala-d-Ala C-terminus of the pentapeptide, thus blocking the addition of late precursors by transglycosylation to the nascent peptidoglycan chain and preventing subsequent cross-linking by transpeptidation (figure 1) [5]. Vancomycin does not penetrate into the cytoplasm; therefore, interaction with its target can take place only after translocation of the precursors to the outer surface of the membrane.

MECHANISM OF RESISTANCE TO VANCOMYCIN

Because vancomycin does not interact with cell wall biosynthetic enzymes but forms complexes with peptidoglycan precursors, its activity is not determined by the affinity for a target enzyme but by the substrate specificity of the enzymes that determine the structure of peptidoglycan precursors. Resistance to vancomycin is due to the presence of operons that encode enzymes (1) for synthesis of low-affinity precursors, in which the C-terminal d-Ala residue is replaced by d-lactate (d-Lac) or d-serine (d-Ser), thus modifying the vancomyin-binding target; and (2) for elimination of the high-affinity precursors that are normally produced by the host, thus removing the vancomycin-binding target [6].

Target modification. VanA-type resistance, which is characterized by inducible high levels of resistance to vancomycin and teicoplanin (table 1), was the first type of resistance described and is mediated by transposon Tn*1546* and elements closely related to it. The transposon encodes a dehydrogenase (VanH), which

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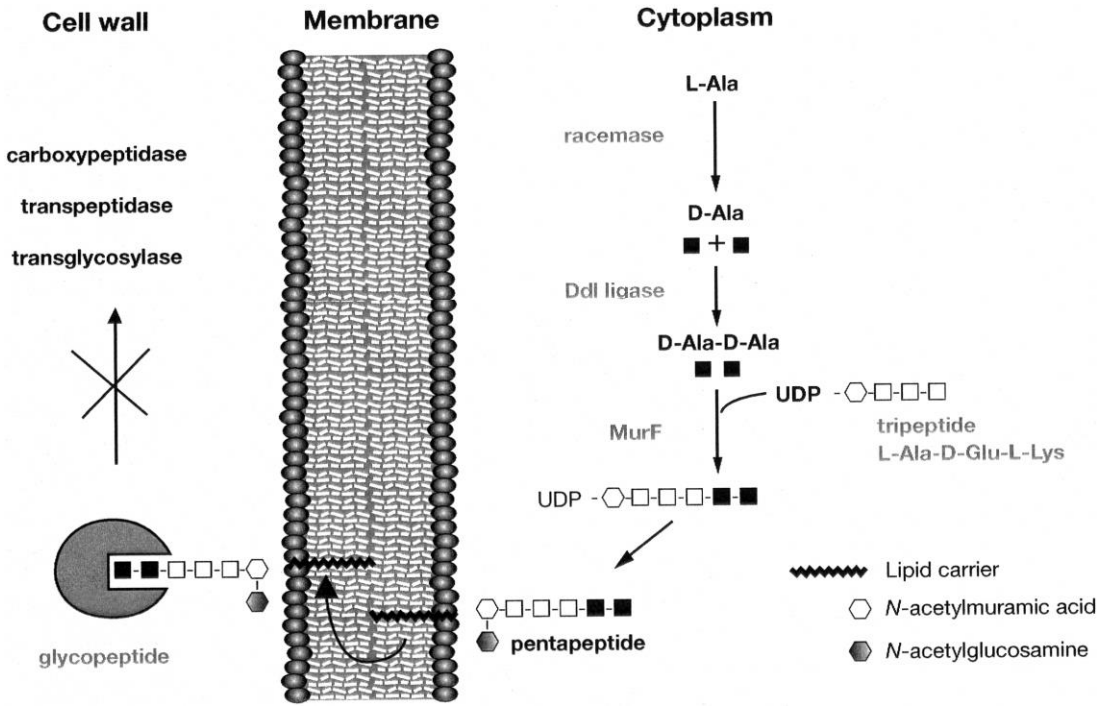

Figure 1. Peptidoglycan biosynthesis and mechanism of action of vancomycin. Binding of the antibiotic to the C-terminal d-Ala–d-Ala of late peptidoglycan precursors prevents reactions catalyzed by transglycosylases, transpeptidases, and the D,D-carboxypeptidases. Ddl, d-Ala:d-Ala ligase; MurF, a synthetase protein; UDP, uracil diphosphate.

reduces pyruvate to d-Lac, and the VanA ligase, which catalyzes the formation of an ester bond between d-Ala and d-Lac (figure 2) [6]. The resulting d-Ala-d-Lac depsipeptide replaces the d-Ala-d-Ala dipeptide in peptidoglycan synthesis, a substitution that decreases the affinity of the molecule for glycopeptides considerably [7].

The VanC resistance phenotype was described first in *Enterococcus gallinarum* [8] and then in the *Enterococcus casseliflavus–Enterococcus flavescens* [9] species, which possess intrinsic low levels of resistance to vancomycin and are susceptible to teicoplanin (table 1). Three genes are required for VanC-type resistance (figure 3): *vanT* encodes the VanT membrane-bound serine racemase, which produces d-Ser; the *vanC* gene product VanC synthesizes d-Ala-d-Ser, which replaces d-Ala-d-Ala in late peptidoglycan precursors; and *vanXYc* encodes the $VanXY_C$ protein, which possesses both D,D-dipeptidase and D,D-carboxypeptidase activities and allows hydrolysis of precursors ending in d-Ala [10]. Substitution of the ultimate d-Ala by a d-Ser results in steric hindrance that reduces its affinity for vancomycin [11].

Removal of the susceptible target. The simultaneous production of precursors ending in d-Ala or d-Lac does not lead

Table 1. Level and type of resistance to vancomycin in enterococci.

Strain characteristic	Acquired resistance level, type					Intrinsic resistance, low level, type VanC1/C2/C3
	High, VanA	Variable, VanB	Moderate, VanD	Low, VanG	Low, VanE	
MIC, mg/L						
Vancomycin	64–100	4–1000	64–128	16	8–32	2–32
Teicoplanin	16–512	0.5–1	4–64	0.5	0.5	0.5–1
Conjugation	Positive	Positive	Negative	Positive	Negative	Negative
Mobile element	Tn*1546*	Tn*1547* or Tn*1549*	…	…	…	…
Expression	Inducible	Inducible	Constitutive	Inducible	Inducible	Constitutive Inducible
Location	Plasmid chromosome	Plasmid chromosome	Chromosome	Chromosome	Chromosome	Chromosome
Modified target	d-Ala-d-Lac	d-Ala-d-Lac	d-Ala-d-Lac	d-Ala-d-Ser	d-Ala-d-Ser	d-Ala-d-Ser

NOTE. d-Ala-d-Lac, d-alanine-d-lactate; d-Ala-d-Ser, d-alanine-d-serine.

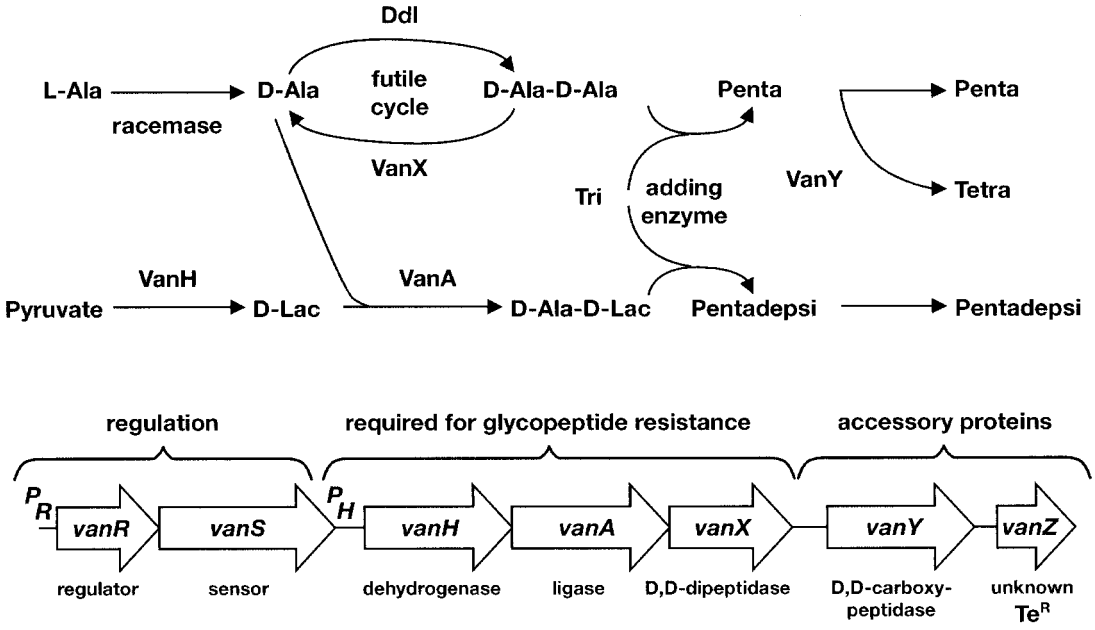

Figure 2. VanA-type glycopeptide resistance. *Top,* Synthesis of peptidoglycan precursors in a VanA-type resistant strain. Ddl, d-Ala:d-Ala ligase; penta, l-Ala-g-d-Glu-l-Lys-d-Ala-d-Ala; Pentadepsi, l-Ala-g-d-Glu-l-Lys-d-Ala-d-Lac; Tetra, l-Ala-g-d-Glu-l-Lys-d-Ala; Tri, l-Ala-g-d-Glu-l-Lys. *Bottom,* Organization of the *vanA* operon. Open arrows represent coding sequences and indicate the direction of transcription. The regulatory and resistance genes are cotranscribed from promoters P_R and P_H, respectively.

to resistance [12]. Under these conditions, binding of glycopeptides to precursors that contain d-Ala-d-Ala inhibits peptidoglycan synthesis. The interaction of vancomycin with its target is prevented by the removal of the susceptible precursors that terminate in d-Ala [13]. Two enzymes are involved in this process (figure 2): the VanX D,D-dipeptidase, which hydrolyzes the d-Ala-d-Ala dipeptide synthesized by the host d-Ala:d-Ala ligase (Ddl) [14], and the VanY D,D-carboxypeptidase, which removes the C-terminal d-Ala residue of late peptidoglycan precursors when elimination of d-Ala-d-Ala by VanX is incomplete [15]. As opposed to VanA-type resistance, in which the VanX and VanY activities are catalyzed by 2 enzymes (figure 2) [15], $VanXY_C$ has both D,D-dipeptidase and D,D-carboxypeptidase activity (figure 3) [16].

Types of resistance. Six types of vancomycin resistance have been characterized on both a phenotypic and a genotypic basis in enterococci (table 1). Five of these types (VanA, B, D, E, and G) correspond to acquired resistance; one type (VanC) is an intrinsic property of *E. gallinarum* and *E. casseliflavus–E. flavescens.* Classification of glycopeptide resistance is currently based on the primary sequence of the structural genes for the resistance ligases rather than on the levels of resistance to glycopeptides, because the MIC ranges of vancomycin and teicoplanin against the various types overlap (table 1). VanA-type strains display high levels of inducible resistance to both vancomycin and teicoplanin, whereas VanB-type strains have variable levels of inducible resistance to vancomycin only [12]. VanD-type strains are characterized by constitutive resistance to moderate levels of the 2 glycopeptides [17]. VanC-, VanE-, and VanG-type strains are resistant to low levels of vancomycin but remain susceptible to teicoplanin [10].

Although the 6 types of resistance involve related enzymatic functions, they can be distinguished by the location of the corresponding genes and by the mode of regulation of gene expression. The *vanA* and *vanB* operons are located on plasmids or in the chromosome [6], whereas the *vanD* [17], *vanC* [18], *vanE* [19], and *vanG* [20] operons have, thus far, been found only in the chromosome.

VanA. VanA is the most frequently encountered type of glycopeptide resistance in enterococci and, to date, is the only one detected in *Staphylococcus aureus* (table 1; figure 2). The prototype Tn*1546* VanA-type resistance element, which was originally detected on a plasmid in an *Enterococcus faecium* clinical isolate, is an 11-kb transposon. It encodes 9 polypeptides that can be assigned to various functional groups: transposition (ORF1 and ORF2), regulation of resistance gene expression (VanR and VanS), synthesis of the d-Ala-d-Lac depsipeptide (VanH and VanA), and hydrolysis of peptidoglycan precursors (VanX and VanY); the function of VanZ remains unknown.

The VanR and VanS proteins are part of a 2-component regulatory system that modulates transcription of the resistance gene cluster [21]. This system is composed of a cytoplasmic VanR response regulator, which acts as a transcriptional activator, and a membrane-bound VanS histidine kinase (figure 4).

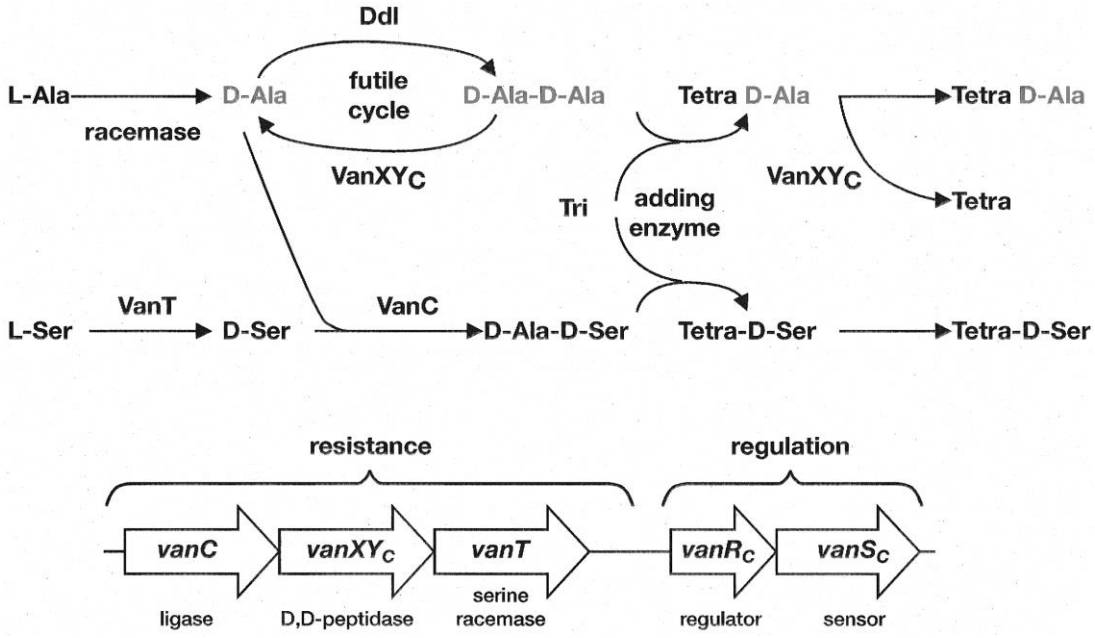

Figure 3. VanC-type glycopeptide resistance. *Top,* Synthesis of peptidoglycan precursors in a VanC-type strain. Ddl, D-Ala:D-Ala ligase; penta, L-Ala-g-D-Glu-L-Lys-D-Ala-D-Ala; Pentadepsi, L-Ala-g-D-Glu-L-Lys-D-Ala-D-Lac; Tetra, L-Ala-g-D-Glu-L-Lys-D-Ala; Tri, L-Ala-g-D-Glu-L-Lys. *Bottom,* Organization of the *vanC* operon. Open arrows represent coding sequences and indicate the direction of transcription.

The *vanA* gene cluster has been found mainly in *E. faecium* and *Enterococcus faecalis* but also in *Enterococcus avium, Enterococcus durans, Enterococcus raffinosus,* and atypical isolates of *E. gallinarum* and *E. casseliflavus,* which are highly resistant to both vancomycin and teicoplanin.

VanB. As in VanA-type strains, acquired VanB-type resistance is due to synthesis of peptidoglycan precursors ending in the depsipeptide D-Ala-D-Lac instead of the dipeptide D-Ala-D-Ala [12]. The organization and functionality of the *vanB* cluster is similar to that of *vanA* but differs in its regulation, because vancomycin, but not teicoplanin, is an inducer of the *vanB* cluster (table 1). The *vanB* operon contains genes encoding a dehydrogenase, a ligase, and a dipeptidase, all of which have a high level of sequence identity (67%–76% identity) with

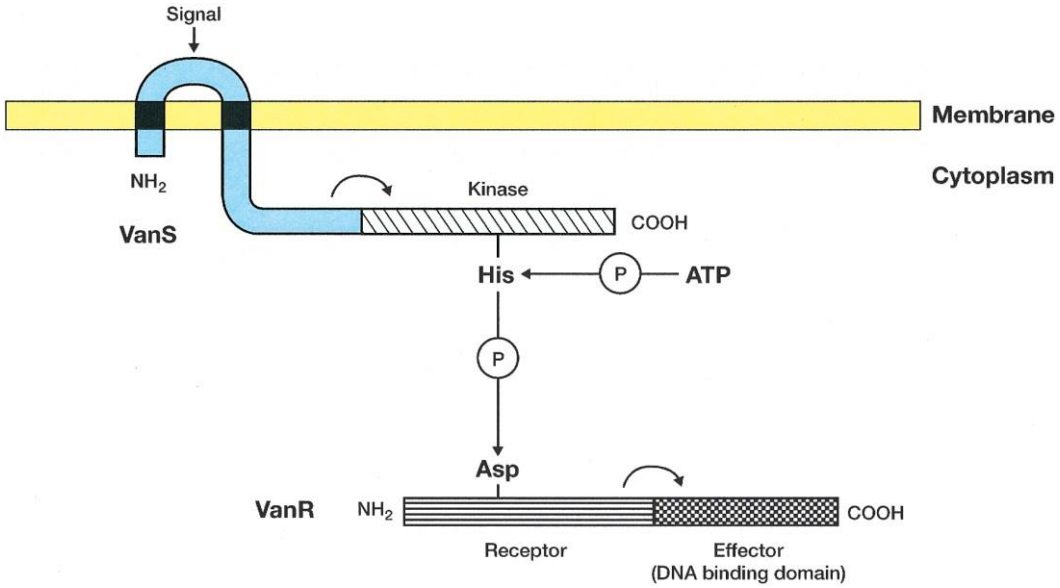

Figure 4. VanRS 2-component regulatory system. Asp, aspartate; His, histidine; P, phosphate.

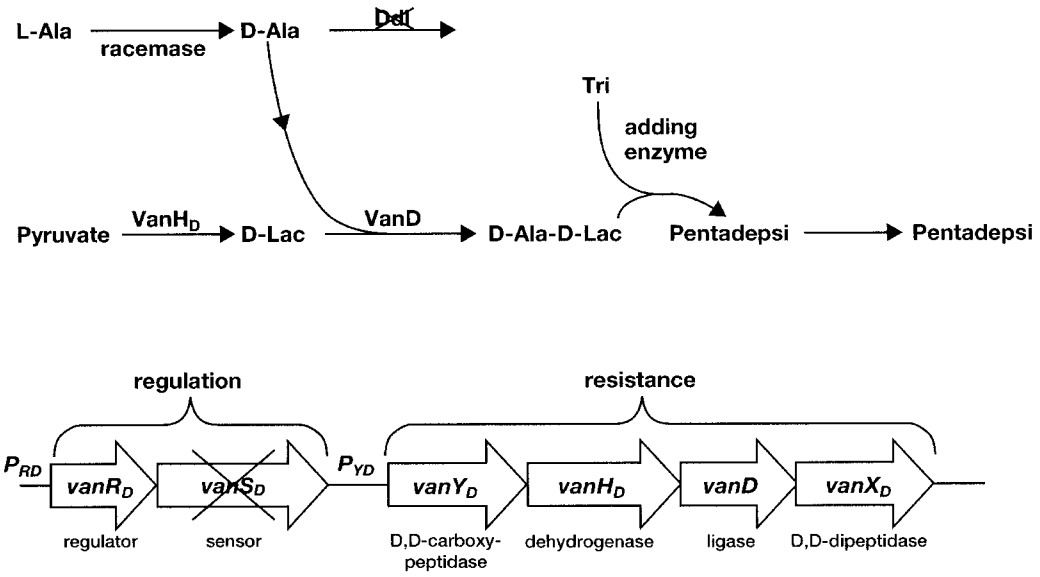

Figure 5. VanD-type glycopeptide resistance. *Top,* Synthesis of peptidoglycan precursors in a VanD-type resistant strain. *Bottom,* Organization of the *vanD* operon. Open arrows represent coding sequences and indicate the direction of transcription. The regulatory and resistance genes are cotranscribed from promoters P_{RD} and P_{YD}, respectively. Ddl, d-alanine:d-alanine ligase; pentadepsi, l-Ala-g-d-Glu-l-Lys-d-Ala-d-Lac; Tri, l-Ala-g-d- Glu-l-Lys; X, mutated *van*S_D nonfunctional gene.

the corresponding deduced proteins of the *vanA* operon and the $vanR_BS_B$ regulatory genes that encode a 2-component system only distantly related to VanRS (34% and 24% identity) [22]. The function of the additional VanW protein found only in the *vanB* cluster is unknown, and there is no gene related to *vanZ*. On the basis of sequence differences, the *vanB* gene cluster can be divided into 3 subtypes: *vanB1*, *vanB2*, and *vanB3* [23, 24]. There is no correlation between the *vanB* subtype and the level of resistance to vancomycin.

VanD. Acquired VanD-type resistance is due to constitutive production of peptidoglycan precursors ending in d-Ala-d-Lac (table 1) [17]. The organization of the *vanD* operon, which is located exclusively in the chromosome in strains that have been studied, is similar to that of *vanA* and *vanB* [17]. However, no genes homologous to *vanZ* or *vanW* from the *vanA* and *vanB* operons, respectively, are present. VanD-type strains share other characteristics that distinguish them from VanA- and VanB-type enterococci. Resistance is constitutive and is not transferable by conjugation to other enterococci [17]. VanD-type strains have negligible D,D-dipeptidase activity, which should result in a susceptible phenotype, because these bacteria are unable to eliminate peptidoglycan precursors ending in d-Ala-d-Ala, which is the target for glycopeptides. However, in VanD-type strains, the susceptible pathway does not function, because the Ddl is inactive as the result of various mutations in the chromosomal *ddl* gene (figure 5) [17, 25]. The gene can be disrupted by a 5-bp insertion, insertion of the IS*19* element, or a point mutation. Consequently, the strains should grow only in the presence of vancomycin, because they rely on the inducible resistance pathway for peptidoglycan synthesis. However, this is not the case because the *vanD* clusters are expressed constitutively as a result of mutations (frameshift or point mutation or insertion-inactivation) in the $VanS_D$ sensor or a point mutation in the $VanR_D$ regulator [17, 25].

Another unusual feature of VanD-type strains is their only slightly diminished susceptibility to teicoplanin (MIC, 4 mg/mL) (table 1) despite their constitutive production of peptidoglycan precursors that terminate mainly in d-Ala-d-Lac. VanD-type strains that constantly activate the *vanD* operon, by mutation in the 2-component regulatory system, and that have eliminated the susceptible pathway, by inactivation of the Ddl, provide a remarkable example of "tinkering" in both intrinsic and acquired genes to achieve higher levels of antibiotic resistance.

VanC. *E. gallinarum* and *E. casseliflavus–E. flavescens* are intrinsically resistant to low levels of vancomycin but remain susceptible to teicoplanin (table 1). The VanC phenotype is expressed constitutively or inducibly as a result of the production of peptidoglycan precursors ending in d-Ser [10]. Three *vanC* genes encoding d-Ala:d-Ser ligases have been described: *vanC-1* in *E. gallinarum*, *vanC-2* in *E. casseliflavus*, and *vanC-3* in *E. flavescens*. The organization of the *vanC* operon (figure 3), which is chromosomally located and is not transferable, is distinct from those of *vanA*, *vanB*, and *vanD*. Three proteins are required for VanC-type resistance: VanT, a membrane-bound serine racemase, which produces d-Ser; VanC, a ligase that catalyzes synthesis of d-Ala-d-Ser; and $VanXY_C$, which possesses both D,D-dipeptidase and D,D-carboxypeptidase activities and allows hydrolysis of precursors ending in d-Ala (figure

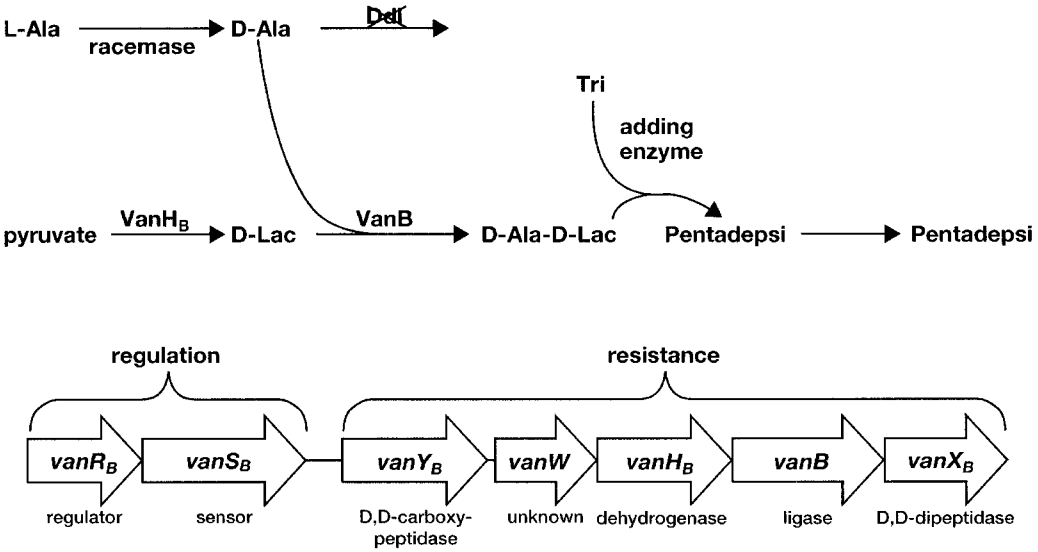

Figure 6. Synthesis of peptidoglycan precursors in a vancomycin-dependent strain. Because of inactivation of the host chromosomal d-Ala:d-Ala ligase (Ddl), the presence of vancomycin in the culture medium is required to induce expression of the resistance pathway, thus allowing cell wall synthesis. Pentadepsi, l-Ala-g-d-Glu-l-Lys-d-Ala-d-Lac; Tri, l-Ala-g-d-Glu-l-Lys.

3). In the *vanA*, *vanB*, and *vanD* clusters, the genes encoding the 2-component regulatory systems (i.e., VanRS, $VanR_BS_B$, or $VanR_DS_D$) are located upstream from the resistance genes (figure 2), whereas, in the *vanC* cluster, these genes are downstream from *vanT* (figure 3). The deduced proteins of the *vanC-2* operon from *E. casseliflavus* display high degrees of identity (71%–91% identity) with those encoded by the *vanC* operon, and those of the *vanC-3* gene cluster from *E. flavescens* display extensive identity with *vanC-2* (97%–100% identity), including the intergenic regions [26]. It is, therefore, difficult to distinguish between *E. casseliflavus* and *E. flavescens* as 2 different species [26].

VanE. The VanE phenotype corresponds to low-level resistance to vancomycin and susceptibility to teicoplanin due to synthesis of peptidoglycan precursors terminating in d-Ala-d-Ser as in intrinsically resistant *Enterococcus* species (table 1). The *vanE* cluster has an organization identical to that of the *vanC* operon [19].

VanG. Acquired VanG type is characterized by resistance to low levels of vancomycin (MIC, 16 mg/mL) but susceptibility to teicoplanin (MIC, 0.5 mg/mL) [27] and by inducible synthesis of peptidoglycan precursors ending in d-Ala-d-Ser (table 1). The chromosomal *vanG* cluster is composed of 7 genes recruited from various *van* operons [20]. In contrast to the other *van* operons, the cluster contains 3 genes *(*$vanU_G$, $vanR_G$, and $vanS_G$*)* encoding a putative regulatory system. $vanR_G$ and $vanS_G$ have the highest similarity to $vanR_D$ and $vanS_D$, and the additional $vanU_G$ gene encodes a predicted transcriptional activator. A protein of this type has not previously been associated with glycopeptide resistance.

Glycopeptide-dependent strains. An intriguing and clinically important phenomenon that has developed in some VanA- and VanB-type enterococci is vancomycin dependence (figure 6). These strains are not only resistant to vancomycin or to both vancomycin and teicoplanin, but also require their presence for growth. Variants of glycopeptide-resistant *E. faecalis* and *E. faecium* that grow only in the presence of glycopeptides have been isolated in vitro, in animal models, and from patients treated with vancomycin for long periods [28, 29]. In the presence of vancomycin, the *vanA*- or *vanB*-encoded d-Ala:d-Lac ligase is induced, which overcomes the defect in synthesis of peptidoglycan precursors ending in d-Ala-d-Ala because of the lack of a functional Ddl following various mutations in the *ddl* gene and, thus, permits growth of the bacteria [29]. Because these strains require particular growth conditions, their prevalence is probably underestimated and not easily detected by routine laboratory testing. Reversion to vancomycin independence has been observed and occurs as a result of a mutation in either the VanS or $VanS_B$ sensor, which leads to constitutive production of d-Ala-d-Lac and, thus, to teicoplanin resistance, or in the *ddl* gene, which restores synthesis of d-Ala-d-Ala and leads to a VanB phenotype inducible by vancomycin [29].

Regulation of resistance. Expression of glycopeptide resistance is regulated by a VanS/VanR-type 2-component signal-transduction system composed of a membrane-bound histidine kinase and a cytoplasmic response regulator that acts as a tran-

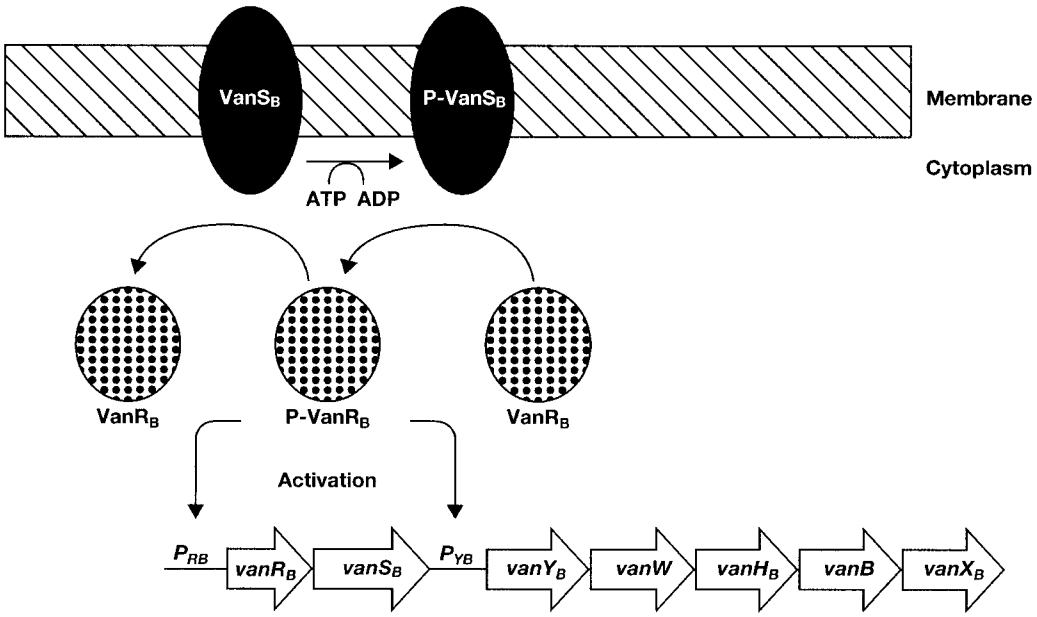

Figure 7. Positive phosphorylation and negative dephosphorylation of the VanR regulator by the VanS$_B$ sensor. Open arrows represent coding sequences and indicate the direction of transcription. The regulatory and resistance genes are cotranscribed from promoters P_{RB} and P_{YB}, respectively. ADP, adenosine diphosphate.

scriptional activator (figure 4) [21]. VanS-type sensors comprise an N-terminal glycopeptide sensor domain with 2 membrane-spanning segments and a C-terminal cytoplasmic kinase domain (figure 4). After a signal associated with the presence of a glycopeptide in the culture medium, the cytoplasmic domain of VanS catalyzes ATP-dependent autophosphorylation on a specific histidine residue and transfers the phosphate group to an aspartate residue of VanR present in the effector domain (figure 7) [21]. VanS also stimulates dephosphorylation of VanR in the absence of glycopeptide [30]. The VanS sensor therefore modulates the phosphorylation level of the VanR regulator: it acts as a phosphatase under noninducing conditions and as a

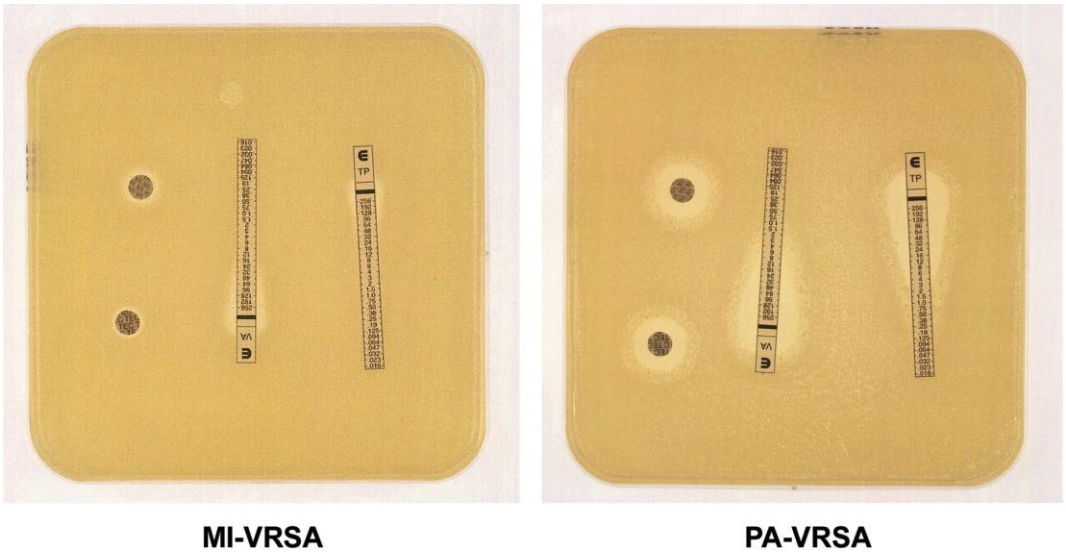

Figure 8. Vancomycin susceptibility determination, by disk diffusion and by E-test, of the Michigan vancomycin-resistant *Staphylococcus aureus* (MI-VRSA) isolate and the Pennsylvania vancomycin-resistant *S. aureus* (PA-VRSA) isolate. In both panels, the upper disk denotes vancomycin, the lower disk denotes teicoplanin, the left strip denotes vancomycin (VA), and the right strip denotes teicoplanin (TP).

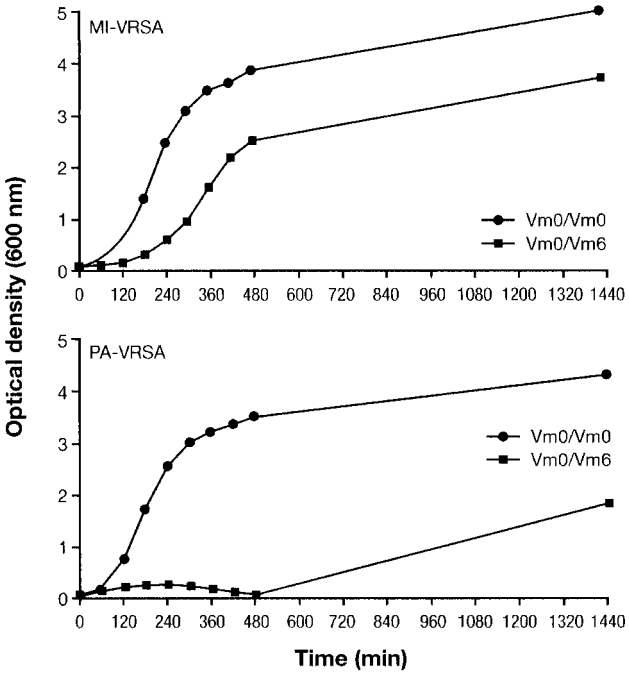

Figure 9. Effect of pregrowth without vancomycin (Vm0) or with 6 mg/mL vancomycin (Vm6) in the culture medium on subsequent growth of the Michigan vancomycin-resistant *Staphylococcus aureus* (MI-VRSA) isolate *(top)* and the Pennsylvania vancomycin-resistant *S. aureus* (PA-VRSA) isolate *(bottom).* The key to the right of each panel shows the vancomycin concentration in the overnight culture/the concentration in the culture medium.

kinase in the presence of glycopeptides, leading to phosphorylation of the response regulator and activation of the resistance genes (figure 7) [30].

GENETICS OF THE *VAN* OPERONS

vanA *Operon.* The *vanA* gene cluster was detected originally on the nonconjugative Tn*1546* transposon [31]. VanA-type resistance in clinical isolates of enterococci is mediated by genetic elements, identical or closely related to Tn*1546*, that are generally carried by self-transferable plasmids [1] and, occasionally, by the host chromosome as part of larger conjugative elements [32]. Tn*1546*-like elements are highly conserved, except for the presence of insertion sequences that have transposed into intergenic regions that are not essential for expression of glycopeptide resistance. Conjugal transfer of plasmids that have acquired Tn*1546*-like elements by transposition appears to be responsible for the spread of glycopeptide resistance in enterococci.

vanB *Operon.* Gene clusters related to *vanB* are generally carried by large (90–250-kb) elements that are transferable by conjugation from chromosome to chromosome [33]. Plasmid-borne *vanB* clusters have also been detected in clinical isolates of enterococci [34]. Much of the dissemination of VanB-type resistance appears to have resulted from the spread of *vanB2* clusters carried on Tn*916*-like conjugative transposons. Two related elements, Tn*5382* (27 kb in size) [35] and Tn*1549* (34 kb in size) [36], have been identified widely in the United States and in Europe.

vanG *Operon.* Transfer of VanG-type resistance is associated with the movement, from chromosome to chromosome, of genetic elements of ~240 kb that also carry *ermB*-encoded erythromycin resistance [20].

***Glycopeptide resistance in* S. aureus.** The transfer of *van* resistance genes from *Enterococcus* species to *S. aureus*, which results in high levels of resistance to vancomycin, was obtained in vitro and in an animal model [37]. Most important, this transfer also occurs in vivo. Recently, 3 methicillin-resistant *S. aureus* (MRSA) isolates with high or moderate levels of resistance to vancomycin and teicoplanin have been isolated from patients in Michigan (MI-VRSA), Pennsylvania (PA-VRSA), and New York after acquisition of the *vanA* gene cluster [38–40].

Clinical isolate MI-VRSA is highly resistant to both glycopeptides [40], whereas PA-VRSA [39] and the New York VRSA strain [38] are moderately resistant to vancomycin and have reduced susceptibility to teicoplanin (figure 8). The 3 isolates harbor a plasmidborne Tn*1546* element [41–43]. A vancomycin-resistant *E. faecalis* strain and a vancomycin-susceptible MRSA strain (MI-MRSA) were isolated from the same patient as MI-VRSA and are considered to be the Tn*1546* donor and recipient, respectively [40]. The vancomycin-resistant *E. faecalis* strain from Michigan harbors a broad-host-range plasmid that contains a copy of Tn*1546* [44], and strain MI-MRSA also contains a resident plasmid. In MI-VRSA, the Tn*1546* element is borne by a plasmid that is identical to the resident plasmid in MI-MRSA except for a copy of Tn*1546*. Therefore, the *Enterococcus* plasmid apparently behaved as a suicide Tn*1546* delivery vector to the plasmid in MI-MRSA [43]. Analysis of the nucleotide sequences flanking Tn*1546* indicated that the transposon was flanked by 5-bp duplications of target DNA [45] typical of the Tn*3* family of elements [46] to which Tn*1546* belongs [31]. This observation confirms that Tn*1546* has transposed into the plasmid of MI-MRSA. Comparative analysis of peptidoglycan precursors and of D,D-dipeptidase (VanX) and D,D-carboxypeptidase (VanY) activities indicated high similar levels of expression of the *vanA* gene clusters in the MI-VRSA and PA-VRSA strains. Thus, the difference in glycopeptide resistance between the 2 isolates is not due to a difference in *van* gene expression.

The stability of the *vanA* operon was studied in MI-VRSA and PA-VRSA by replica plating. No vancomycin-susceptible clones of MI-VRSA were obtained, whereas ~50% of the PA-VRSA derivatives were susceptible after overnight growth in the absence of vancomycin. In MI-VRSA, transposition of Tn*1546* from the enterococcal to the resident plasmid rescued the incoming genetic information, whereas, in PA-VRSA, gly-

copeptide resistance is due to the acquisition of a large plasmid, presumably also from *Enterococcus* species, that probably does not replicate efficiently in the new host.

The effect of prior induction by vancomycin on growth of the MI-VRSA and PA-VRSA isolates indicated that, as opposed to MI-VRSA, when PA-VRSA was grown overnight in the absence or presence of antibiotic and was subcultured with vancomycin, growth was delayed for a minimum of 8 h (figure 9). Taken together, these data suggest that low-level vancomycin resistance of the PA-VRSA strain, and maybe also of the New York VRSA strain, could be due to a longer delay in induction of resistance associated with a high rate of spontaneous loss of the vancomycin resistance determinant or to a low rate of loss of a resistance trait with high biological cost [45].

CONCLUSIONS

Major advances in the understanding of the biochemical mechanisms and genetics of vancomycin resistance in enterococci have been achieved. However, the origin of the resistance genes remains unclear. The glycopeptide-producing organisms, which have to protect themselves against the products of their secondary metabolism, represent a potential source for resistance because they harbor genes encoding homologues of VanS, VanR, VanH, VanA, and VanX [47]. Alternately, the vancomycin-resistant biopesticide *Paenibacillus popilliae*, which contains the *vanF* operon related to the *vanA* cluster, could be the progenitor of the resistance genes acquired by enterococci [48].

Glycopeptides, alone or in combination, often constitute the only therapy for infection with multiresistant strains of staphylococci, streptococci, and enterococci. The emergence and dissemination of high-level resistance to vancomycin in enterococci can lead to clinical isolates resistant to all antibiotics. Although enterococci are not highly pathogenic, the incidence of vancomycin resistance among clinical isolates is steadily increasing, and such isolates have become important as nosocomial pathogens and as a reservoir of resistance genes. Dissemination of glycopeptide resistance to more pathogenic bacteria, such as staphylococci and streptococci, has occurred, because there is no barrier to heterospecific expression or gene transfer among gram-positive cocci. Such a transfer in nature may be underestimated because of low-level phenotypic expression in the new host. The 16-year delay in detection of VanA-type resistance in *S. aureus* and its apparent rarity could be due to inefficient replication of enterococcal plasmids in staphylococci. Thus, the efficacy of glycopeptide resistance transfer from *Enterococcus* species to *Staphylococcus* species, similar to the transfer to gram-negative bacteria [49], results from the combined multiplicative probabilities of the transfer process and of the mechanism of stabilization of exogenous DNA. The frequency of the second event is very low, because transposons of the Tn*3* family, Tn*1546* in particular [31], are very stable genetic elements.

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