Journal of Bacteriology and Virology 2015. Vol. 45, No. 2 p.67 – 78
http://dx.doi.org/10.4167/jbv.2015.45.2.67

Review Article

Glycopeptide Antibiotics: Structure and Mechanisms of Action

Hee-Kyoung Kang[1] and Yoonkyung Park[1,2*]

[1]Department of Biomedical Sciences, Chosun University, Gwangju; [2]Research Center for Proteinaceous Materials, Chosun University, Gwangju, Korea

Glycopeptides of the clinically important antibiotic drugs are glycosylated cyclic or polycyclic nonribosomal peptides. Glycopeptides such as vancomycin and teicoplanin are often used for the treatment of gram-positive bacteria in patients. The increased incidence of drug resistance and inadequacy of these therapeutics against gram-positive bacterial infections would be the formation and clinical development of more variable second generation of glycopeptide antibiotics: semisynthetic lipoglycopeptide analogs such as telavancin, dalbavancin, and oritavancin with improved activity and better pharmacokinetic properties. In this review, we describe the development of and bacterial resistance to vancomycin, teicoplanin, and semisynthetic glycopeptides (teicoplanin, dalbavancin, and oritavancin). The clinical influence of resistance to glycopeptides, particularly vancomycin, are also discussed.

Key Words: Glycopeptide; Resistance; Vancomycin; Teicoplanin; Dalbavancin

INTRODUCTION

Glycopeptides are the most prevalent class of therapeutics that are used for the treatment against as severe infections caused by Gram-positive pathogens, such as enterococci, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Clostridium difficile*. Since the 20th century, the emergence of vancomycin-resistant enterococci (VRE) and vancomycin-resistant *S. aureus* (VRSA) in the presents the new infectious disease challenge to public health when few new drugs including glycopeptide antibiotics are being developed. In the 1990's, the emergence of resistance to vancomycin, first among enterococci (such as *Enterococcus faecium* and *E. faecalis*) and then among *S. aureus* (vancomycin-intermediate *S. aureus*, VISA or glycopeptide-intermediate *S. aureus*, GISA) has caused researchers to develop the second-generation glycopeptides and caused a flurry of activity targeted at understanding the mechanisms of bacterial resistance and the evolution of glycopeptide antibiotics (1, 2).

Glycopeptides are glycosylated cyclic or polycyclic nonribosomal peptides produced by a various group of filamentous actinomycetes. These therapeutics target gram-positive bacteria by binding to the acyl-D-Ala-D-Ala terminus to the growing peptidoglycan and then cross-linking peptides within and between peptidoglycan on the outer surface of the cytoplasmic membrane (3). Glycopeptide-resistant bacteria avoid such a fate by replacing the D-Ala-D-Ala C-terminus of the pentapeptide with D-Ala-D-Lac or D-Ala-D-Ser, thus

Received: April 7, 2015/ Revised: April 18, 2015/ Accepted: April 20, 2015
*Corresponding author: Yoonkyung Park. Department of Biomedical Sciences and Research Center for Proteinaceous Materials, Chosun University, Gwangju 501-759, Korea.
Phone: +82-62-230-6854, Fax: +82-62-225-6758, e-mail: y_k_park@chosun.ac.kr
**This work was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korean Government (MEST; No. 2011-0017532) and the Global Research Laboratory (GRL; NRF-2014K1A1A2064460).

changing the glycopeptide-binding target and for removal of the high-affinity precursors that eliminating the glycopeptide-binding target. The antimicrobial resistance has manifestation in enterococci and staphylococci via the expression of *van* gene clusters encoding proteins that reprogram cell wall synthesis and thereby prevent the action of these glycopeptide antibiotics (4). These mechanisms of antimicrobial resistance were easily co-opted from glycopeptide producer actinomycetes, which use them to prevent self-harm when producing antibiotics (these mechanisms were less likely to be orchestrated by the pathogenic bacteria after prolonged treatment). Some *van*-like gene clusters with a high level of homology and an organization similar to those described in enterococci, were identified in many glycopeptide-producing actinomycetes, such as *Amycolatopsis* spp., which produces vancomycin, *Actinoplanes teichomyceticus* ATCC 31121, which produces teicoplanin, and *Streptomyces toyocaensis*, which produces the A47934 glycopeptide, but an understanding of their active function in affecting resistance is only at the beginning (4, 5).

In this review, we describe the current understanding of the mechanisms of action and bacterial resistance to the natural and semisynthetic glycopeptides.

CHEMICAL STRUCTURE OF NATURAL GLYCOPEPTIDES

Glycopeptide antibiotics are often used to treat life-threatening infections by multi-drug-resistant gram-positive organisms, such as *S. aureus*, *Enterococcus* spp., and *Clostridium difficile*. They are drugs of final resort against MRSA, which is these days a leading cause of community-acquired infections and results in high morbidity and death rates among patients with hospital-acquired infections (4, 6). Natural glycopeptides composed of a cyclic peptide core comprised of seven amino acids, to which two aminosugars are bound to the amino acid core. Binding of this type of antibiotic to its target (D-Ala-D-Ala terminal end of peptidoglycan precursors) complexes via a set of five hydrogen bonds with the peptidic backbone of the therapeutic agent. The presence of the chlorine or sugar moiety in oritavancin facilitates homo-dimerization, allowing for stronger interactions to the target site (7, 8). A lipophilic side chain (present in teicoplanin and in all the semisynthetic glycopeptides) have been proposed to bind to bacterial membrane. It increases antibacterial potency and prolongs half-life (Fig. 1).

Vancomycin, produced by the actinomycete *Amycolatopsis orientalis*, was first introduced into clinical practice in 1958 (6). Vancomycin contains proteinogenic (Tyr, Leu, Asn, Ala, and Glu) and nonproteinogenic amino acid residues (4-hydroxyphenylglycine, 3,5-dihydroxyphenylglycine, and β-hydroxytyrosine). Five of the seven residues in vancomycin are aromatic, and two are aliphatic amino acids. Whereupon, three of oxidative cross-links between aromatic amino acid residues results in a peculiar structural conformation: a binding pocket for the cellular antibiotic target (1, 9). In the 1980s, MRSA, coagulase-negative staphylococci, and enterococci emerged as resistant pathogens and aroused a renewed clinical interest in vancomycin. The increased use was accompanied by emergence of resistance first among enterococci and subsequently among staphylococci, menacing the subsequent utility of vancomycin and vancomycin-like glycopeptides (4, 9).

Teicoplanin is a glycopeptide antibiotic that is produced by the actinomycete *Actinoplanes teichomyceticus* (10). Teicoplanin and vancomycin have a similar mode of many chemical and microbiological properties, but teicoplanin has longer elimination half-life and the possibility of administration by intramuscular injection. However, resistance is much more common among coagulase-negative staphylococci, and the recommended doses of the antibiotic may be too low for more severe cases of infection (11). Teicoplanin is a complex of six analogous molecules. As in vancomycin, the core aglycone is a cyclic heptapeptide backbone consisting of aromatic amino acid residues and carries two sugar moieties D-mannose and *N*-acetyl-β-D-glucosamine and a fatty-acid chain (12). The fatty-acid component increases teicoplanin's lipophilicity, resulting in greater cellular and tissue penetration (4, 12).

Glycopeptide antibiotics inhibit synthesis of the bacterial cell wall by binding to the dipeptide terminus D-Ala-D-Ala

of peptidoglycan precursors, thereby sequestering the substrate from transpeptidation and transglycosylation reactions at the late extracellular stages of peptidoglycan cross-linking. The complex of D-Ala-D-Ala with glycopeptides is stabilized by an arrange of hydrophobic van der Waals bonds and five hydrogen bonds lining the antibiotic-binding pocket (1, 13). Cross-linked peptidoglycans are needed for sufficient tensile strength of the cell wall. Thus, a glycopeptide's action finally destabilizes the cell wall, and the bacterial cell death occurs presumably due to osmotic damage. The necessity of the direct access for glycopeptides to the target peptidoglycan precursor explains the selective action against gram-positive organisms. Such bacteria have peptidoglycan precursors on the surface of the cytoplasmic membrane, whereas gram-negative bacteria are protected by the outer lipopolysaccharide membrane impermeable to large biomolecules and hydrophobic compounds from the environment (14).

The spectrum of teicoplanin's activity against gram-positive bacteria is similar to that of vancomycin, but teicoplanin has greater potency, particularly against some clinical microbe of the genera *Staphylococcus*, *Streptococcus*, and *Enterococcus* (4, 14). Consequently, most of the semisynthetic glycopeptides were created by introducing hydrophobic moieties into the heptapeptide scaffold to ensure the membrane-anchoring ability, thus leading to more effective drugs (4, 9, 15).

SEMISYNTHETIC GLYCOPEPTIDES

Semisynthetic glycopeptides telavancin, oritavancin, and dalbavancin have been developed to overcome the emergence of MRSA strains showing weaken sensitivity to vancomycin and to increase the penetration into tissues and into cerebrospinal fluid. These new molecules are lipoglycopeptides and are characterized by longer half-life in comparison with vancomycin; these semisynthetic glycopeptides may prove improvements for infrequent dosing and results in greater potency and lower risk of development of resistant microorganisms (4, 9, 16).

Telavancin is a derivative of vancomycin and differs from the parent compound by the presence of an additional hydrophobic side chain on the vancomsamine sugar and hydrophilic phosphonate group (17) (Fig. 1). Accordingly, compared to vancomycin or oritavancin, telavancin possesses specific properties, multiple modes of action, including alterations of membrane integrity (oritavancin's mechanism), and protrusively shorter half-life, although it is strongly protein bound and largely distributes in the living organism (18, 19). Hydrophilic properties of the negatively charged phosphonate group significantly improve adsorption, distribution, metabolism, and the excretion profile of telavancin. Pharmacological researches suggest that the increased antimicrobial action of telavancin (compared to that of vancomycin) on *Streptococcus pneumoniae*, *S. aureus*, and enterococci including VRE results from a complex mechanism of action, which involves disorderes in lipid synthesis and membrane disintegration (9, 15, 20).

Oritavancin is an *N*-alkyl-*p*-chlorophenyl-benzyl derivative of the natural glycopeptide chloroeremomycin produced by the actinomycete *Amycolatopsis orientalis* (21) (Fig. 1). The chlorophenyl-benzyl side chain is accountable to the prolonged half-life of oritavancin and probably for the strong antimicrobacterial effects of this compound because this side chain allows for anchoring in and subsequent disruption of the cell membrane (22). The additional epivancosamine group promotes formation of dimers, which cooperatively bind to precursors of peptidoglycans (containing terminal D-Ala-D-Ala or D-Ala-D-Lac), and may elucidate the residual activity against vancomycin-resistant bacteria. Although, in general, oritavancin has a spectrum of activity comparable to that of vancomycin, it offers marked advantages in terms of intrinsic bactericidal activity especially against streptococci, and its effectiveness is not affected by the antibiotic-resistance mechanisms developed by staphylococci and enterococci; oritavancin also kills *C. difficile* (23). According to recent studies on the mode of action, the biaryl group is responsible for cell membrane depolarization. The superior activity against gram-positive pathogens, including those resistant to vancomycin, is because of this dual mechanism of action: either inhibition of cell wall biosynthesis or disruption of membrane integrity (1,

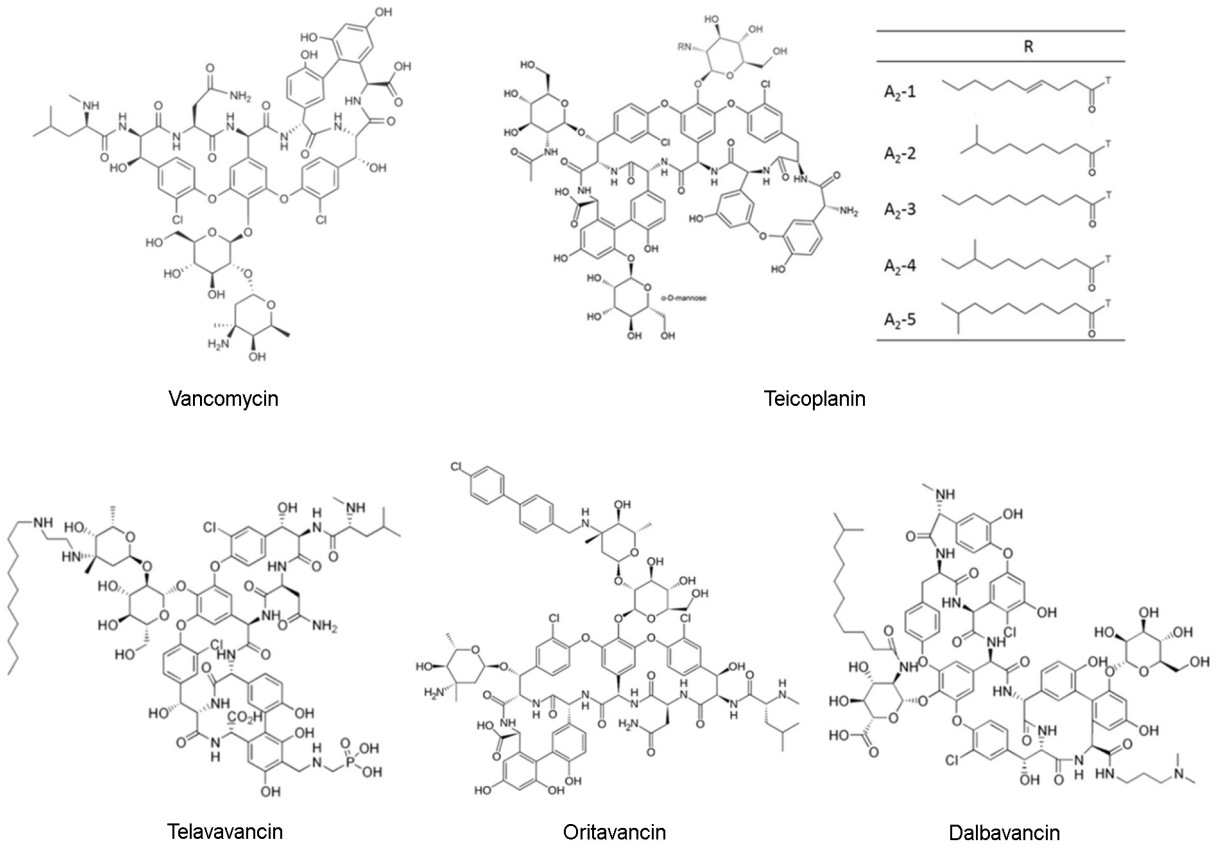

Figure 1. The chemical structure of glycopeptides. Vancomycin and teicoplanin are natural products. In teicoplanin, A_2-1 through A_2-5 denote the components of the complex that are characterized by a fatty-acid moiety at position R. Oritavancin and telavancin are semisynthetic second-generation glycopeptides from the vancomycin family. Dalbavancin is a semisynthetic derivative of teicoplanin.

12, 21, 23).

Dalbavancin is a semisynthetic antibiotic derived from a teicoplanin analog (A-40926) via modification of the functional groups and sugar moieties of A-40926, without disruption of the D-Ala-D-Ala-binding site, which is required for antimicrobial activity (24) (Fig. 1). It is a lipoglycopeptide compound with di-[3-demethylaminopropyl]amide, *N*-alkylated on the aminoglucoronyl moiety. Dalbavancin prevents the synthesis of the bacterial cell wall by binding to the D-Ala-D-Ala residues of growing peptidoglycan chains and thus inhibits disrupts peptidoglycan elongation and cell membrane formation. Compared to vancomycin, dalbavancin shows a very potent *in vitro* activity against the majority of gram-positive pathogenic bacteria as well as long half-life (6~10 days), allowing for once-weekly intravenous dosing. Dalbavancin's antibacterial activity is similar to that of teicoplanin but with lower minimum inhibitory concentrations (MICs; Table 1). Dalbavancin is not the most effective in this group of antibiotics but shows the best tolerability (4, 15, 20, 24).

The success of these three semisynthetic glycopeptides as therapeutic candidates and their enhanced antibacterial properties (in comparison with vancomycin) are stimulating further efforts to study the mechanisms of action/resistance and to develop better derivatives (1, 16). The novel tailoring enzymes discovered by the Brady group in arrayed metagenomic libraries represent a successful strategy for creation of libraries of glycopeptide antibiotic variants (4, 25).

THE MECHANISM OF ACTION

The bacterial cell wall contains a rigid or semi-rigid envelope lying outside the cell membrane called peptidoglycan, or murein, which provides structural support. Peptidoglycan monomers made up of sugar backbone with peptide and disaccharide units that are attached by glycosidic bonds into long chains via transglycosidation. The glycopeptide antibiotics can pass through the cell membrane to the site of polymerization, where they form noncovalent bonds with the terminal carbohydrates, in an action that finally inhibits the cross-linking by the trans-peptidase. Subsequently, the weakened cell wall can no longer hold up the positive osmotic pressure within the cell; this situation results in cytolysis and death of the bacterial cell (16).

Vancomycin acts by interfering with the synthesis of the cell wall in gram-positive bacteria. Because of the variety of mechanisms by which gram-negative bacteria produce their cell wall and the various factors that affect penetration of the outer membrane of gram-negative bacteria, vancomycin is not active against such bacteria (except for some nongonococcal species of *Neisseria*). The primary target of vancomycin is the D-Ala-D-Ala terminus of pentapeptidic precursors; empirical studies and molecular modeling (9, 26) indicate that vancomycin forms the complex with the D-Ala-D-Ala residues by forming five hydrogen bounds with the peptide backbone of the glycopeptide. This complex prevents the transpeptidation reactions via steric hindrance. Recent studies showed the importance of the protonated state of vancomycin and of the formation of dimers of glycopeptide antibiotic molecules during this interaction (27).

Teicoplanin inhibits cell wall synthesis in susceptible microbes. It inhibits the synthesis of peptidoglycans in the bacterial cell wall by the nonspecific binding and the saturation of the outer layers of bacterial peptidoglycans. Teicoplanin then binds to the D-Ala-D-Ala terminus of the precursors, which fits into a cleft in the teicoplanin molecule (3). The antibiotic activity spectrum of teicoplanin, like that of vancomycin, is restricted to aerobic and anaerobic gram-positive bacteria. The bactericidal profiles of the two agents are not identical: teicoplanin is generally more active than vancomycin against gram-positive bacteria including *Streptococci*; the two agents show similar activity against *S. aureus*, including MRSA; however, teicoplanin is less active against some strains of coagulase-negative staphylococci. Inoculum size influences the activity of teicoplanin, and variable bactericidal activity against some strains of coagulase-negative staphylococci have been observed with the type of testing culture media. Stimultaneous resistance to teicoplanin and vancomycin is difficult to lead under laboratory conditions, and the small increase in resistance that may develop is lost when the bacteria are subcultured in the absence of the drugs (9, 14).

Oritavancin's improved inhibition of cell wall peptidoglycan synthesis may be ascribed to a cooperative binding to the target of pentapeptide side chain; this mechanism is possible because of the ability of the oritavancin molecule to dimerize (15, 21). The increased steric hindrance around peptidoglycan precursors is caused by the presence of a bulky substituent on its disaccharide moiety; this mechanism allows for potent inhibition of both transglycosylation and transpeptidation steps in the peptidoglycan biosynthesis (15, 21). The 4'-chlorobiphenylmethyl group allowed for the disruption of the cell membrane of gram-positive bacteria. Moreover, oritavancin shows a rapid antibacterial effect on vancomycin-sensitive and vancomycin-resistant staphylococci (in the exponential and stationary phases) as well as on biofilm-producing bacteria; these effects proceed simultaneously with membrane permeabilization and membrane depolarization, which is the most favorable promoted by the anchoring of the lipophilic side chain of oritavancin in the cell membrane (21, 28).

Telavancin has a dual mechanism of action with both inhibition of peptidoglycan biosynthesis and membrane depolarization. It acts by binding to the peptidoglycan precursor called "lipid (undecaprenyl)-linked *N*-acetylglucosamine-*N*-muramylpentapeptide" at the D-Ala-D-Ala residues. This interaction inhibits transglycosylation (peptidoglycan polymerization) and the final transpeptidation (cross-linking) steps. Telavancin is a strong inhibitor of peptido-

glycan biosynthesis at the specific transglycosylase and shows a 10-fold greater effectiveness (than does vancomycin) at inhibiting the peptidoglycan biosynthesis in intact MRSA cells (16, 29). The decylaminoethyl hydrophobic side chain promotes interaction with the cell membrane, and this interaction improves the binding affinity for peptidoglycan intermediates at the target site in the bacterial cell surface. Telavancin also lead to rapid concentration-dependent reduction of the membrane potential. The mechanism of action seemed to be involves the interaction with peptidoglycan intermediates (15, 16, 29). This phenomenon may take place via binding to lipid intermediate II molecules and telavancin, which disrupts both peptidoglycan synthesis and membrane barrier function. This second mode of action is specific for bacterial cell membranes, not mammalian cells, and appears to cause to the more rapid antibacterial activity of telavancin compared to that of vancomycin (15, 29).

Telavancin differs from vancomycin in that the majority of the molecules are associated with the cell membrane integrity rather than the cell wall biosynthesis. This dual mode of binding promotes both the interaction of the carboxylate binding pocket with terminal D-Ala-D-Ala residues and interaction of the decylaminoethyl side chain with the bacterial cell membrane (15, 16, 19).

Dalbavancin is a lipoglycopeptide from the same glycopeptide class as vancomycin. Just as other glycopeptides, dalbavancin exerts its antimicrobial effect by disrupting cell well biosynthesis. Dalbavancin's mechanism of action is similar to that of other glycopeptide antibiotics: it interferes with the transpeptidation and transglycosylation step in cell wall synthesis by binding to the D-Ala-D-Ala carboxyl terminus of a stem pentapeptide in an incipient peptidoglycan; this action is typical for gram-positive bacteria. The binding of dalbavancin to this substrate inhibits the cross-linking reactions that provide the bacterial cell wall its rigidity and strength. Dalbavancin also dimerizes and anchors itself in a lipophilic bacterial membrane, thereby enhancing its stability in the target condition and its affinity for peptidoglycans. This increased inter action with the bacterial cell wall contributes to dalbavancin's pharmacokinetic and pharmacodynamic properties, specifically its extended half-life (20, 30).

MECHANISMS OF BACTERIAL RESISTANCE

Resistance to glycopeptides among enterococci is mediated by acquirement of a gene operon located in a floating genetic element that codes for concerted production of enzymes involved in the synthesis of low-affinity peptidoglycan precursors (with terminal D-Ala-D-Lac or D-Ala-D-Ser) and in the removal of high-affinity peptidoglycan precursors (with terminal D-Ala-D-Ala); such a resistance-inducing operon may instead encode a regulatory system permitting for induction by glycopeptides (31). Nine types of vancomycin resistance have been documented from the phenotypic and genotypic standpoint. Table 1 summarizes their main features regarding location and transferability of the operon, regulation of the expression, transcription of the *van*A-N gene, and the level of resistance to vancomycin and other glycopeptide antibiotics (32~40).

The VanA type of resistance is the most widespread and most reported to date; it is characterized by acquired inducible resistance to both vancomycin and teicoplanin (32~34). Three enzymes are necessary for resistance to glycopeptides, namely, D-Lac dehydrogenase VanH, which converts pyruvate to D-Lac; ligase VanA, which produces formation of an ester bond between D-Ala and D-Lac instead of the usual D-Ala-D-Ala; and D-Ala-D-Ala dipeptidase VanX, which hydrolyze a residual D-Ala-D-Ala dipeptide, but does not recognize D-Ala-D-Lac (41, 42). Two accessory enzymes can increase the level of resistance. Via an unknown mechanism, VanZ confers weak resistance to teicoplanin in the absence of the other resistance-related proteins (43, 44). VanY is encodes a D,D-carboxypeptidase that hydrolyses the C-terminal D-Ala residue of the pentapeptide synthesized by means of the D-Ala-D-Ala dipeptides that escaped VanX hydrolysis (45, 46). A two-component regulatory system, consisting of the membrane-bound histidine kinase sensor protein VanS and the cytoplasmic regulator protein VanR (which acts as a transcriptional activator) allows for induction of the operon's transcription after exposure to

Table 1. Types of resistance to vancomycin and teicoplanin among enterococci, in relation to alternative peptidoglycans

Glycopeptide -resistant phenotype	Microorganism	Resistance level	MIC (mg/mL)		Location of *van* genes	Transcription of genes	C-terminus of modified target	Reference
			Vancomycin	Teicoplanin				
VanA	*E. faecalis* *E. faecium*	High	64~100	16~512	Plasmid Chromosome	Inducible	D-Ala-D-Lac	32~34
VanB	*E. faecalis* *E. faecium*	Variable	4~1,000	0.5~1.0	Plasmid Chromosome	Inducible	D-Ala-D-Lac	34~36
VanC	*E. gallinarum* *E. casseliflavus* *E. flavescens*	Low intrinsic level	2~32	0.5~1.0	Chromosome	Constitutive	D-Ala-D-D-Ser	35, 36, 43
VanD	*E. faecalis* *E. faecium*	Moderate	64~128	6~64	Chromosome	Constitutive	D-Ala-D-Lac	35, 37
VanE	*E. faecalis*	Low	8~32	0.5	Chromosome	Inducible	D-Ala-D-Ser	35
VanG	*E. faecalis* *E. faecium*	Low	16	0.5	Chromosome	Inducible	D-Ala-D-Ser	35
VanL	*E. faecalis*	Low	8	Susceptible	Chromosome	Inducible	D-Ala-D-Ser	36
VanM	*E. faecium*	Variable	>256	0.75	Plasmid Chromosome	Inducible	D-Ala-D-Lac	38
VanN	*E. faecium*	Low	16	0.5	Chromosome	Constitutive	D-Ala-D-Ser	39, 40

MIC: minimal inhibitory concentration.

glycopeptides. In turn, the activated VanR binds to DNA and induces expression of VanH, VanA and VanX (43, 44). The molecule responsible for inducing VanS dimerization and activation has been a subject of intensive research: it is still debated whether VanS dimerization and activation are caused by the direct binding of glycopeptides to VanS or its activation of binding an intermediate in cell wall biosynthesis (that accumulate as a result of antibiotic action) (47). The sensor kinase (called VanSB) of VanB-type enterococci responds to different signals, in contrast to VanS, which is activated by vancomycin but not activated by teicoplanin. Actually, vancomycin and teicoplanin induce resistance among VanA enterococci, whereas VanB-type enterococci are sensitive to vancomycin but resistant to teicoplanin (31, 34, 44) (Table 1).

Because VanA, VanB, and VanD phenotypes of resistance result from the preferential incorporation of D-Ala and D-Lac-ending peptidoglycan precursors, the three phenotypes are different in their inducibility, antibicrobial specificity, and in the level of resistance (Table 1, Fig. 2). More significant differences exist among VanC, VanE, and VanG types of resistance: the ligase produces D-Ala-D-Ser less than D-Ala-D-Lac. Therefore, VanT, a membrane-bound serine racemase replaces the dehydrogenase VanH (48). Moreover, the VanXY protein, which has a bi-functional D,D-dipeptidase/D,D-carboxypeptidase activies, replaces VanX (D-Ala-D-Ala peptidase) and VanY (D,D-carboxypeptidase) and allows for hydrolysis of ending in D-Ala peptidoglycan precursors (Fig. 2, 31, 43, 44).

The mechanism of moderate resistance among staphylococci (vancomycin-intermediate *S. aureus*, VISA) is multifactorial and is not yet entirely understood. This global scale analysis of gene and protein expression uncovered a series of proteins or genes overexpressed in resistant strains; these proteins are usually global regulator attenuator, or hypermutability factors (24). Their role in the resistance phenotype requires to be further researched. VISA strains show decreased growth rates and an increased thickness of the outer cell wall than fully susceptible strains (49). Both VISA and hVISA (heterogeneous VISA) produce three- to

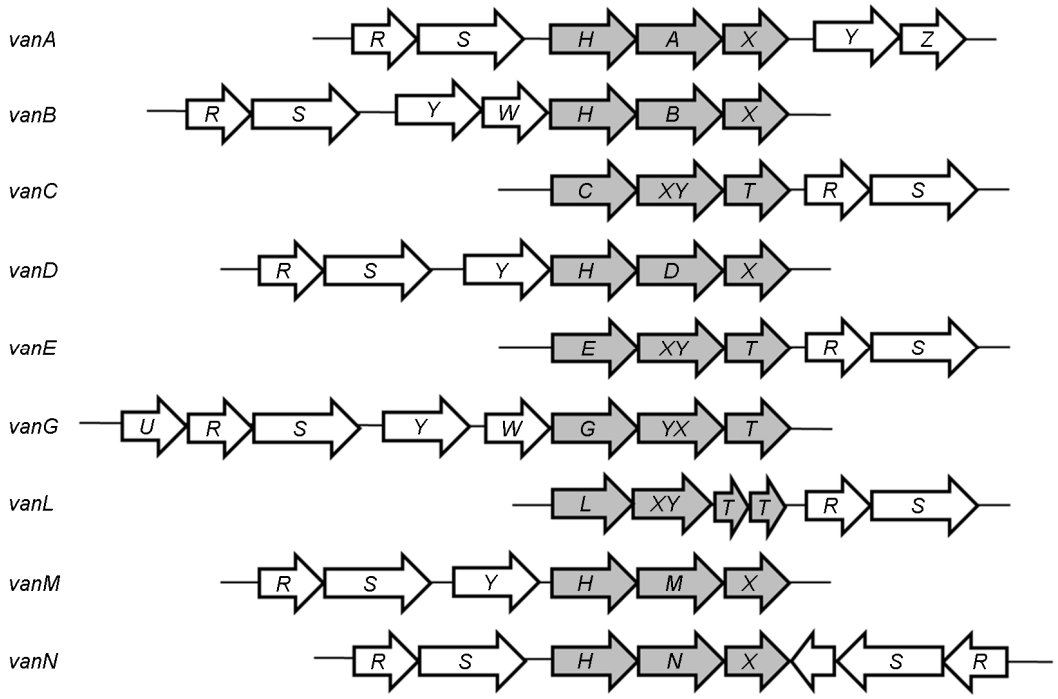

Figure 2. Alignment of *van* resistance gene clusters from glycopeptide antibiotics-producing bacteria. Arrows indicate the direction of transcription. Empty arrow indicate hypothetical gene. A to N represent the D-Ala-D-Lac ligase giving name to the gene cluster. U, transcription regulator; R, regulator; S, histidine kinase; H, dehydrogenase; Y, D,D-carboxypeptidase; W and Z, unknown protein; vanXY, D,D-carboxypeptidase/D,D-dipeptidase; T, serine recemase.

five-fold increased levels of penicillin-binding proteins 2 and 2' and of cell wall precursors (50). In contrast to hVISA, VISA shows increased amount of glutamine nonamidated muropeptides in cell wall; this process decreases the cross-linking within the cell wall and increases the amount of vancomycin bound to the peptidoglycan precursors. This mechanism worsen the ability of vancomycin to reach the bacterial cell surface, where primary targets of this antibiotic are situated (51). Unlike VRSA isolates, strains of VISA or hVISA do not carry vancomycin resistance genes such as v*anA*, *vanB*, or *vanC* (52). Although the mechanism has not been conclusively determined for VISA or hVISA, many hypothetical mechanisms such as defects in DNA mismatch repair have been proposed (31, 53). The VISA phenotype acquisition has been probably a multistep process and occurs due to changes in the peptidoglycan synthesis process. VISA strains have been reported to synthesize excessive amounts of D-Ala-D-Ala (54). The extra layers of cell wall precursors prevent vancomycin molecules to reach their target sites. One important difference between VRSA and hVISA is that a decrease in glycopeptide selective pressure in the environment may reduce VRSA predominance. hVISA, however, has been reported to prevail even in the absence of glycopeptide pressure (31, 55).

In addition, emergence of the VISA phenotype is associated with functional loss of the accessory gene regulator *agr* operon and with *agr* II polymorphism (56). The role of *agr* in the VISA phenotype is not yet known, but *agr* is known to coordinately control the expression of exotoxins, exoproteins, and components of adhesion points; *agr* mutants and VISA strains show decreased autolysis and virulence *in vitro* (56, 57). It is noteworthy that vancomycin failure in patients has been associated with *agr* group II (56).

VRSA strains is due to acquisition of the VanA gene cluster by conjugative transfer of high-level vancomycin resistance from enterococci to *S. aureus*. Although some of

these strains have high vancomycin resistance (MIC ≥32 mg/l), others do not. This phenomenon is thought to be related to the stability of the antibiotic resistance genes after the transfer (58). This finding was alarming because *S. aureus* is responsible for severe infections and toxicoses both in hospitals and in the community, and for almost three decades, vancomycin has been increasingly used to treat *S. aureus* infections because of the worldwide emergence of MRSA, which is multiple drugs resistant (12).

Lipoglycopeptides offer only partial alleviation of the treatment resistance. Dalbavancin's effectiveness is affected by the teicoplanin resistance mechanism. Telavancin shows improved *in vitro* activity retained against VISA, but MICs of VRSA or VRE, though lower than those against vancomycin, remain high. Oritavancin is the most effective glycopeptide against VRSA and VRE, probably because of its ability to form dimeric structure that can bind with higher affinity to modified peptidoglycan precursors and at lower concentrations than vancomycin (59). Resistance has not developed to oritavancin among *S. aureus* strains including VISA, but VanA and VanB strains of enterococci with decreased sensitivity to oritavancin have been developed *in vitro*. Dalbavancin has been shown *in vitro* activity against methicillin-sensitive *S. aureus*, MRSA, VISA, methicillin-resistant *S. epidermidis*, and against enterococcal strains. But it has poor activity against vancomycin-resistant (vanA) enterococci and VRSA (4, 15, 31). This lack of activity against VRE strains that contain the vanA gene differentiates dalbavancin from the other investigational glycopeptides, oritavancin and telavancin.

CONCLUSION

Significant advances have been achieved in the field of glycopeptide-antibiotic research, particularly in the past 20 years. This progress is necessary considering the significant challenge of vancomycin resistance faced by the medical community. Vancomycin is the member of the class of reliable and critically available glycopeptide antibiotics against severe infections with β-lactam-resistant gram-positive bacteria. On the other hand, emergence, spread, and environmental effect of antimicrobial resistance to vancomycin (and to other glycopeptide agents like teicoplanin) among clinical gram-positive cocci (e.g., the *Enterococcus* species, *S. aureus*, and coagulase-negative staphylococci) have made it hard to handle serious infections caused by such gram-positive pathogens. It is necessary to look for alternatives such as vancomycin and other glycopeptides for the treatment of severe infections caused by gram-positive microorganisms. The development of semisynthetic glycopeptides that mechanistically address the inherent resistance resulting from either inducible or constitutive peptidoglycan remodeling of the pentapeptide chain terminating in D-Ala-D-Lac is an urgent task. Such an accomplishment is expected to not only solve the appearance problem of acquired bacterial resistance but also offer a new class of more effective glycopeptide antibiotics on nature's designs.

REFERENCES

1) James RC, Pierce JG, Okano A, Xie J, Boger DL. Redesign of glycopeptide antibiotics: back to the future. ACS Chem Biol 2012;7:797-804.
2) Butler MS, Hansford KA, Blaskovich MA, Halai R, Cooper MA. Glycopeptide antibiotics: back to the future. J Antibiot (Tokyo) 2014;67:631-44.
3) Reynolds PE. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur J Clin Microbiol Infect Dis 1989;8:943-50.
4) Binda E, Marinelli F, Marcone GL. Old and New glycopeptide antibiotics: action and resistance. Antibiotics 2014;3:572-94.
5) Marcone GL, Binda E, Carrano L, Bibb M, Marinelli F. Relationship between glycopeptide production and resistance in the actinomycete *Nonomuraea* sp. ATCC 39727. Antimicrob Agents Chemother 2014;58:5191-201.
6) Rossolini GM, Arena F, Pollini S. Novel infectious diseases and emerging gram-positive multiresistant pathogens in hospital and community acquired infections. In Antimicrobials; Marinelli F, Genilloud O, editors. 1st ed. Berlin Heidelberg:Springer Verlag, 2014.
7) Treviño J, Bayón C, Ardá A, Marinelli F, Gandolfi R,

Molinari F, *et al*. New insights into glycopeptide antibiotic binding to cell wall precursors using SPR and NMR spectroscopy. Chemistry 2014;20:7363-72.
8) Allen NE, Nicas TI. Mechanism of action of oritavancin and related glycopeptide antibiotics. FEMS Microbiol Rev 2003;26:511-32.
9) Van Bambeke F, Van Laethem Y, Courvalin P, Tulkens PM. Glycopeptide antibiotics: from conventional molecules to new derivatives. Drugs 2004;64:913-36.
10) Parenti F, Beretta G, Berti M, Arioli V. Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. I. Description of the producer strain, fermentation studies and biological properties. J Antibiot (Tokyo) 1978;31:276-83.
11) Sharma V, Jindal N. *In vitro* activity of vancomycin and teicoplanin against coagulase negative staphylococci. Oman Med J 2011;26:186-8.
12) Marcone GL, Marinelli F. Glycopeptides: An old but up to date successful antibiotic class. In Antimicrobials; Marinelli F, Genilloud O, editors. 1st ed. Berlin Heidelberg:Springer Verlag, 2014.
13) Cooper MA, Williams DH. Binding of glycopeptide antibiotics to a model of a vancomycin-resistant bacterium. Chem Biol 1999;6:891-9.
14) Kahne D, Leimkuhler C, Lu W, Walsh C. Glycopeptide and lipoglycopeptide antibiotics. Chem Rev 2005;105: 425-48.
15) Zhanel GG, Calic D, Schweizer F, Zelenitsky S, Adam H, Lagacé-Wiens PR, *et al*. New lipoglycopeptides: A comparative review of dalbavancin, oritavancin and telavancin. Drugs 2010;70:859-86.
16) Ashford PA, Bew SP. Recent advances in the synthesis of new glycopeptide antibiotics. Chem Soc Rev 2012; 41:957-78.
17) Leadbetter MR, Adams SM, Bazzini B, Fatheree PR, Karr DE, Krause KM, *et al*. Hydrophobic vancomycin derivatives with improved ADME properties: discovery of telavancin (TD-6424). J Antibiot (Tokyo) 2004;57: 326-36.
18) Shaw JP, Seroogy J, Kaniga K, Higgins DL, Kitt M, Barriere S. Pharmacokinetics, serum inhibitory and bactericidal activity, and safety of telavancin in healthy subjects. Antimicrob Agents Chemother 2005;49:195 -201.
19) Sun HK, Duchin K, Nightingale CH, Shaw JP, Seroogy J, Nicolau DP. Tissue penetration of telavancin after intravenous administration in healthy subjects. Antimicrob Agents Chemother 2006;50:788-90.
20) Zhanel GG, Trapp S, Gin AS, DeCorby M, Lagacé-Wiens PR, Rubinstein E, *et al*. Dalbavancin and telavancin: novel lipoglycopeptides for the treatment of Gram-positive infections. Expert Rev Anti Infect Ther 2008;6:67-81.
21) Das B, Sarkar C, Schachter J. Oritavancin. - a new semisynthetic lipoglycopeptide agent to tackle the challenge of resistant gram positive pathogens. Pak J Pharm Sci 2013;26:1045-55.
22) Zhanel GG, Schweizer F, Karlowsky JA. Oritavancin: mechanism of action. Clin Infect Dis 2012;54:S214-9.
23) Belley A, McKay GA, Arhin FF, Sarmiento I, Beaulieu S, Fadhil I, *et al*. Oritavancin disrupts membrane integrity of *Staphylococcus aureus* and vancomycin-resistant enterococci to effect rapid bacterial killing. Antimicrob Agents Chemother 2010;54:5369-71.
24) Malabarba A, Goldstein BP. Origin, structure, and activity *in vitro* and *in vivo* of dalbavancin. J Antimicrob Chemother 2005;55:ii15-20.
25) Banik JJ, Brady SF. Recent application of metagenomic approaches toward the discovery of antimicrobials and other bioactive small molecules. Curr Opin Microbiol 2010;13:603-9.
26) Loll PJ, Axelsen PH. The structural biology of molecular recognition by vancomycin. Annu Rev Biophys Biomol Struct 2000;29:265-89.
27) Yang Z, Vorpagel ER, Laskin J. Influence of the charge state on the structures and interactions of vancomycin antibiotics with cell-wall analogue peptides: experimental and theoretical studies. Chemistry 2009;15:2081-90.
28) Belley A, Neesham-Grenon E, McKay G, Arhin FF, Harris R, Beveridge T, *et al*. Oritavancin kills stationary-phase and biofilm *Staphylococcus aureus* cells *in vitro*. Antimicrob Agents Chemother 2009;53:918-25.
29) Saravolatz LD, Stein GE, Johnson LB. Telavancin: a novel lipoglycopeptide. Clin Infect Dis 2009;49:1908 -14.
30) Bennett JW, Lewis JS, Ellis MW. Dalbavancin in the treatment of complicated skin and soft-tissue infections: a review. Ther Clin Risk Manag 2008;4:31-40.

31) Sujatha S, Praharaj I. Glycopeptide resistance in gram-positive cocci: a review. Interdiscip Perspect Infect Dis 2012;2012:781679.
32) Arthur M, Quintiliani R Jr. Regulation of VanA- and VanB-type glycopeptide resistance in enterococci. Antimicrob Agents Chemother 2001;45:375-81.
33) Wright GD, Holman TR, Walsh CT. Purification and characterization of VanR and the cytosolic domain of VanS: a two-component regulatory system required for vancomycin resistance in *Enterococcus faecium* BM-4147. Biochemistry 1993;32:5057-63.
34) Healy VL, Lessard IA, Roper DI, Knox JR, Walsh CT. Vancomycin resistance in enterococci: reprogramming of the D-Ala-D-Ala ligases in bacterial peptidoglycan biosynthesis. Chem Biol 2000;7:R109-19.
35) Courvalin P. Vancomycin resistance in gram-positive cocci. Clin Infect Dis 2006;42:S25-34.
36) Arthur M, Reynolds P, Courvalin P. Glycopeptide resistance in enterococci. Trends Microbiol 1996;4:401-7.
37) Depardieu F, Kolbert M, Pruul H, Bell J, Courvalin P. VanD-type vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. Antimicrob Agents Chemother 2004;48:3892-904.
38) Xu X, Lin D, Yan G, Ye X, Wu S, Guo Y, *et al*. *vanM*, a new glycopeptide resistance gene cluster found in *Enterococcus faecium*. Antimicrob Agents Chemother 2010;54:4643-7.
39) Lebreton F, Depardieu F, Bourdon N, Fines-Guyon M, Berger P, Camiade S, *et al*. D-Ala-D-Ser VanN-type transferable vancomycin resistance in *Enterococcus faecium*. Antimicrob Agents Chemother 2011;55:4606-12.
40) Nomura T, Tanimoto K, Shibayama K, Arakawa Y, Fujimoto S, Ike Y, *et al*. Identification of VanN-type vancomycin resistance in an *Enterococcus faecium* isolate from chicken meat in Japan. Antimicrob Agents Chemother 2012;56:6389-92.
41) Bugg TD, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. Biochemistry 1991;30:10408-15.
42) Bugg TD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Identification of vancomycin resistance protein VanA as a D-alanine:D-alanine ligase of altered substrate specificity. Biochemistry 1991;30:2017-21.
43) Depardieu F, Podglajen I, Leclercq R, Collatz E, Courvalin P. Modes and modulations of antibiotic resistance gene expression. Clin Microbiol Rev 2007;20:79-114.
44) Yim G, Thaker MN, Koteva K, Wright G. Glycopeptide antibiotic biosynthesis. J Antibiot (Tokyo) 2014;67:31-41.
45) Arthur M, Depardieu F, Snaith HA, Reynolds PE, Courvalin P. Contribution of VanY D,D-carboxypeptidase to glycopeptide resistance in *Enterococcus faecalis* by hydrolysis of peptidoglycan precursors. Antimicrob Agents Chemother 1994;38:1899-903.
46) Arthur M, Depardieu F, Molinas C, Reynolds P, Courvalin P. The *vanZ* gene of Tn*1546* from *Enterococcus faecium* BM4147 confers resistance to teicoplanin. Gene 1995;154:87-92.
47) Arthur M, Molinas C, Courvalin P. The VanS-VanR two-component regulatory system controls synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J Bacteriol 1992;174:2582-91.
48) Podmore AH, Reynolds PE. Purification and characterization of VanXY(C), a D,D-dipeptidase/D,D-carboxypeptidase in vancomycin-resistant *Enterococcus gallinarum* BM4174. Eur J Biochem 2002;269:2740-6.
49) Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunn B, *et al*. Emergence of vancomycin resistance in *Staphylococcus aureus*. glycopeptide-intermediate *Staphylococcus aureus* working group. N Engl J Med 1999;340:493-501.
50) Biavasco F, Manso E, Varaldo PE. *In vitro* activities of ramoplanin and four glycopeptide antibiotics against clinical isolates of *Clostridium difficile*. Antimicrob Agents Chemother 1991;35:195-7.
51) Renzoni A, Kelley WL, Vaudaux P, Cheung AL, Lew DP. Exploring innate glycopeptide resistance mechanisms in *Staphylococcus aureus*. Trends Microbiol 2010;18:55-6.
52) Finan JE, Archer GL, Pucci MJ, Climo MW. Role of penicillin-binding protein 4 in expression of vancomycin resistance among clinical isolates of oxacillin-resistant

Staphylococcus aureus. Antimicrob Agents Chemother 2001;45:3070-5.

53) Muthaiyan A, Jayaswal RK, Wilkinson BJ. Intact *mutS* in laboratory-derived and clinical glycopeptide-intermediate *Staphylococcus aureus* strains. Antimicrob Agents Chemother 2004;48:623-5.

54) Appelbaum PC. The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 2006;12:16-23.

55) Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev 2010;23:99-139.

56) Sakoulas G, Eliopoulos GM, Fowler VG Jr, Moellering RC Jr, Novick RP, Lucindo N, *et al*. Reduced susceptibility of *Staphylococcus aureus* to vancomycin and platelet microbicidal protein correlates with defective autolysis and loss of accessory gene regulator (*agr*) function. Antimicrob Agents Chemother 2005;49:2687-92.

57) Peleg AY, Monga D, Pillai S, Mylonakis E, Moellering Jr RC, Eliopoulos GM. Reduced susceptibility to vancomycin influences pathogenicity in *Staphylococcus aureus* infection. J Infect Dis 2009;199:532-6.

58) Périchon B, Courvalin P. Heterologous expression of the enterococcal *vanA* operon in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2004;48:4281-5.

59) Van Bambeke F, Mingeot-Leclercq MP, Struelens MJ, Tulkens PM. The bacterial envelope as a target for novel anti-MRSA antibiotics. Trends Pharmacol Sci 2008;29:124-34.