Approved Glycopeptide Antibacterial Drugs: Mechanism of Action and Resistance

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The glycopeptide antimicrobials are a group of natural product and semisynthetic glycosylated peptides that show antibacterial activity against Gram-positive organisms through inhibition of cell-wall synthesis. This is achieved primarily through binding to the D-alanyl-D-alanine terminus of the lipid II bacterial cell-wall precursor, preventing cross-linking of the peptidoglycan layer. Vancomycin is the foundational member of the class, showing both clinical longevity and a still preferential role in the therapy of methicillin-resistant *Staphylococcus aureus* and of susceptible *Enterococcus* spp. Newer lipoglycopeptide derivatives (telavancin, dalbavancin, and oritavancin) were designed in a targeted fashion to increase antibacterial activity, in some cases through secondary mechanisms of action. Resistance to the glycopeptides emerged in delayed fashion and occurs via a spectrum of chromosome- and plasmid-associated elements that lead to structural alteration of the bacterial cell-wall precursor substrates.

The glycopeptide antibiotics are a group of glycosylated cyclic or polycyclic nonribosomal peptides that inhibit Gram-positive bacterial cell-wall synthesis. Unlike other antimicrobial classes, these agents act as substrate binders (of cell-wall precursors) as opposed to active-

site enzyme inhibitors (Barna and Williams 1984; Nagarajan 1994). Significant members of this class include vancomycin and the lipoglycopeptide teicoplanin, along with the semisynthetic lipoglycopeptide derivatives telavancin, dalbavancin, and oritavancin (Fig. 1). All

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Figure 1. Glycopeptide and lipoglycopeptide antibacterial structures. Key structural features are highlighted in red.

act to prevent cross-linking of the bacterial cell-wall peptidoglycan layer by binding to the stoichiometrically limited D-alanyl-D-alanine terminus of the lipid II monomer. Although the lipid II substrate is broadly expressed in most bacterial species, the antibacterial spectrum of this class is limited to Gram-positive pathogens. The physiochemical properties of the glycopeptide structure preclude transit through the outer membrane in Gram-negative species, thus blocking access to the lipid II target.

STRUCTURE AND ORIGIN OF GLYCOPEPTIDE AGENTS

Vancomycin (from the root word “vanquish”) is the oldest glycopeptide antibiotic still in clinical use and has been named to the World Health Organization model list of essential medicines (see who.int/medicines/publications/essentialmedicines/EML2015_8-May-15.pdf). A natural product, vancomycin was isolated in 1953 from a strain of *Amycolatopsis orientalis* found in a soil sample (Nagarajan 1994; Kahne et al. 2005; Levine 2006) and first used clinically in 1955 with Food and Drug Administration (FDA) approval to treat penicillin-resistant staphylococci (McCormick et al. 1956). The glycopeptide class name derives from vancomycin’s sugar-decorated heptapeptide structure, the hallmark of which is a central, highly cross-linked, multiring core that includes the polar uncharged amino acid asparagine and an amino-terminal N-methylleucine (Fig. 1) (Harris and Harris 1982). Other amino acid positions are variable across species, differentiating many of the other natural product glycopeptides that have not been pursued as drug candidates (Nagarajan 1994). Also common across these agents is the terminal vancosamine moiety of the disaccharide substituent.

The related natural product, teicoplanin, was isolated ~30 years later from *Actinoplanes teichomyceticus* (Nagarajan 1994) and possesses a hydrophobic substituent that markedly differentiates its properties from vancomycin. Because of this difference, teicoplanin has been termed a lipoglycopeptide. Subsequent advances

in medicinal and organic chemistry have yielded novel semisynthetic lipoglycopeptide derivatives of teicoplanin, vancomycin, and related compounds that show improved activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and are bactericidal for enterococci. Specifically, N-alkyl modifications of the amino sugars and alteration of the terminal carboxyl by amide derivatization (Fig. 1) improve antimicrobial activity of these agents without directly reducing lipid II binding (Allen et al. 1997; Leadbetter et al. 2004; Wenzler and Rodvold 2015).

Such clinically relevant lipoglycopeptide derivatives include the second-generation agents dalbavancin (BI-387) and oritavancin (LY-333328), and the third-generation agent telavancin (TD-6424) (Judice and Pace 2003; Pace et al. 2003; Zhanel et al. 2010; Karlowsky et al. 2015; Leuthener et al. 2015). Dalbavancin is derived structurally from teicoplanin; the addition of an extended, lipophilic side-chain results in enhanced potency and an extended half-life, whereas an amidated carboxyl side group results in enhanced antistaphylococcal activity (Fig. 1) (Smith et al. 2015). Oritavancin is a synthetic derivative of the naturally occurring glycopeptide chloroeremomycin (A82846B) (Nagarajan 1994; Linsdell et al. 1996). The addition of a 4'-chlorobiphenylmethyl substituent to the disaccharide sugar, along with the additional monosaccharide moiety (4-epivancosamine) attached to the amino acid residue on ring 6, confers significantly enhanced activity against vancomycin-resistant enterococci, and vancomycin-intermediate and -resistant staphylococci (Mitra et al. 2015). Telavancin was derived from N-decylaminoethylvancomycin by regioselective reductive alkylation of the vancosamine nitrogen. For telavancin, the addition of the negatively charged, auxiliary hydrophilic group methylaminophosphonic acid to the resorcinol position results in a substantial increase in urinary clearance and decrease in kidney and liver distribution when compared with the parent derivative, but the newer agent also shows diminished bactericidal activity versus the original precursor (Leadbetter et al. 2004; JL Pace, unpubl.).

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ANTIBACTERIAL SPECTRUM

Vancomycin possesses both in vitro and in vivo antibacterial activity against the clinically significant pathogens *Staphylococcus* spp., *Streptococcus* spp., susceptible *Enterococcus* spp., and many lesser species (Table 1). It is also bactericidal against *S. aureus* (including MRSA isolates) and the streptococci. This bactericidal activity led to the increased prevalence of vancomycin use as the incidence of multidrug-resistant MRSA burgeoned clinically, particularly before the approval of newer effective agents such as linezolid and the next-generation lipoglycopeptide agents. Because the physiochemical nature of the glyco- and lipoglycopeptide agents results in limited to no oral bioavailability, vancomycin has been restricted to parenteral administration, with the exception of oral (intraluminal) therapy against *Clostridium difficile* colitis.

Teicoplanin, which has been considered for development in this country but is currently only approved outside of the United States for clinical use, also possesses a Gram-positive antibacterial spectrum. However, in addition to the staphylococci, streptococci, other less signif-

icant species, and vancomycin-susceptible enterococci, teicoplanin shows antibacterial activity against VanB (vancomycin-resistant) enterococci. The spectrum of the semisynthetic derivative dalbavancin is similar (Zhanel et al. 2010). In contrast, oritavancin and telavancin also show markedly improved activity against VanA enterococci, albeit of questionable clinical significance (Zhanel et al. 2010; Arias et al. 2012).

MECHANISMS OF ACTION

Early investigations into glycopeptide activity showed vancomycin-mediated inhibition of amino acid incorporation into bacterial peptidoglycan, with intracellular accumulation of metabolic precursors including the lipid carrier-linked *N*-acetyl-muramic pentapeptide (MurNAc-pentapeptide; lipid II) (Anderson et al. 1965; Chatterjee and Perkins 1966; Sinha and Neuhaus 1968; Perkins 1969). This suggested interference with the transglycosylation step of peptidoglycan assembly. Distribution studies with iodinated vancomycin showed that binding was surface-localized in the bacterial cell

Table 1. Glycopeptide and lipoglycopeptide antibacterial spectrum

Species	MIC ₅₀ /MIC ₉₀ (μg/mL), ECOFF, or breakpoint				
	Vancomycin	Teicoplanin	Telavancin	Oritavancin	Dalbavancin
<i>Staphylococcus aureus</i>	1/1 ^a	0.5/1 ^b	0.06/0.06 ^c	0.03/0.06 ^d	0.06/0.06 ^e
<i>S. aureus</i> (MRSA)	1/2 ^a	1/2 ^b	0.06/0.06 ^c	0.03/0.06 ^d	0.06/0.06 ^e
<i>S. aureus</i> (VISA)	4–8 ^f	2 ^g	0.12/0.25 ^c	–/1 ^h	0.5/– ^e
<i>Streptococcus pneumoniae</i>	0.25/0.5 ^a	0.12/0.12 ^b	0.008/0.015 ^c	0.002/0.004 ^a	0.015/0.03 ^a
<i>Streptococcus pyogenes</i>	0.5/1 ^a	0.5 ^g	0.03/0.03 ^c	0.03/0.25 ^d	≤0.03/≤0.03 ^e
<i>Streptococcus agalactiae</i>	0.25/0.5 ^a	0.12/0.12 ^b	0.03/0.06 ^c	0.03/0.12 ^d	≤0.03/0.12 ^e
<i>Streptococcus anginosus</i>		0.12/0.12 ^b		≤0.008/0.015 ^d	≤0.03/≤0.03 ^e
<i>Enterococcus faecium</i> VSE	0.5/1 ^a	0.25/1 ^b	≤0.015/0.03 ^c	≤0.008/0.015 ^d	0.06/0.12 ^e
<i>E. faecium</i> VRE	512/512 ^a	64/≥64 ⁱ	1/≥1 ^c	0.008/0.06 ^d	≥4/≥4 ^e

VRE, Vancomycin-resistant *Enterococcus*; VSE, vancomycin-susceptible *Enterococcus*.

^aData from Zhanel et al. 2010.

^bData from Gales et al. 2005.

^cData from Karlowsky et al. 2015.

^dData from Mitra et al. 2015.

^eData from Smith et al. 2015.

^fData from CLSI 2016.

^gData from EUCAST 2015.

^hData from Das et al. 2013.

ⁱData from Shonekan et al. 1997.

(Bordet and Perkins 1970; Perkins and Nieto 1970; Nieto and Perkins 1971). Further, it was observed that vancomycin complexed with lipid II, and that peptidoglycan synthesis in *Gaffkya homari* (which uses a distinct, tetrapeptide-linked precursor) was not inhibited (Hammes and Neuhaus 1974). Subsequently, it was concluded that vancomycin binds to the D-alanyl-D-alanine terminating pentapeptide moiety of lipid II, preventing both transpeptidation through substrate sequestration and transglycosylation via associated steric hindrance. The binding of vancomycin to the D-alanyl-D-alanine terminating pentapeptide is mediated by hydrogen binding (Table 2) (Cristofaro et al. 1995; Cooper et al. 1997, 2000; Cooper and Williams 1999; Walsh and Howe 2002).

In addition to the very distinct mechanism of substrate sequestration mediating its antibacterial properties, vancomycin's activity is influenced by noncovalent self-association of the antibiotic molecules (Gerhard et al. 1993; Mackay et al. 1994b; Beauregard et al. 1995; Westwell et al. 1996). Using a variety of antibiotic analogues and substrate proxies as models, the dimerization of vancomycin was determined to be promoted in part by interaction with the lipid II target (Mackay et al. 1994a; Sharman et al. 1997; Loll et al. 1998, 2009). This antibiotic self-association is believed to be entropically beneficial and actually results in orders of magnitude greater affinity, as binding to a second lipid II moiety then requires lower energy because of colocalization (Williams et al. 1993, 1998; Rao et al. 1999a,b; Rekharsky et al. 2006). Cocrystallization studies with vancomycin and substrate target confirm a conformation of what

are termed back-to-back dimers (vancosamine–vancosamine [V–V]); in addition, there can be association of a third vancomycin molecule in a side–side configuration (Williamson and Williams 1981; Schaefer et al. 1996; Loll et al. 1997; Nitani et al. 2009). Studies with synthetic, covalently linked dimers further support these conclusions, with the V–V constructs showing the broadest in vitro antibacterial activity, often superior to that of vancomycin itself (Sundram et al. 1996; Rao et al. 1998; Stack et al. 1998; Staroske and Williams 1998; Arimoto et al. 1999; Nicolau et al. 2001a,b; Griffin et al. 2003; Shiozawa et al. 2003; Li and Xu 2005).

Vancomycin also antagonizes peptidoglycan remodeling, or “autolysis” (Sieradzki and Tomasz 1997; Peschel et al. 2000). The importance of this antagonism may explain the lack of bactericidal activity against otherwise susceptible enterococci, as these species show a low autolytic capacity. Almost certainly, the balance of effects on peptidoglycan synthesis (anabolism) and remodeling (autolysis, catabolism) is critical to antibacterial success for cell-wall inhibitors like the glycopeptides. This mechanism also likely mediates the concentration-independent bactericidal nature of vancomycin against the staphylococci; in fact, with some of the synthetic, covalently linked, vancomycin dimer analogues, treatment of bacteria with higher antibiotic concentrations leads to greater numbers of surviving cells (JL Pace, unpubl.).

Teicoplanin, like vancomycin, mediates its antibacterial activity by binding to the D-alanyl-D-alanine moiety and sequestration of the lipid II substrate, resulting in inhibition of bacterial peptidoglycan synthesis (Barna et al.

Table 2. Glycopeptide and lipoglycopeptide mechanism(s) of action

Antibiotic	Mechanism			
	Lipid II-binding		Transpeptidation (enzymatic)	Membrane (permeabilization–depolarization)
	Dimerization	Membrane anchoring		
Vancomycin	Yes	No	No	No
Teicoplanin	No	Yes	No	No
Dalbavancin	No	Yes	No	No
Oritavancin	Yes	Yes	Yes	Yes
Telavancin	Yes	Yes	Unknown	Yes

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1985; Nagarajan 1994; Beauregard et al. 1995). Importantly, distinct structural differences impact the self-associated dimer formation that is important for vancomycin target affinity but absent with teicoplanin. In contrast, a second evolutionarily beneficial property has been imparted to teicoplanin, which minimizes the lack of dimerization potential (Barna et al. 1985; Beauregard et al. 1995). The hydrophobic moiety of teicoplanin interacts with the lipid bilayer of the bacterial membrane resulting in localization (anchoring) in proximity to the lipid II substrate, thus also providing a potentially energetically favorable interaction that mediates antibacterial action (Barna et al. 1985; Beauregard et al. 1995; Economou et al. 2013). Likewise, dalbavancin mechanism of action is believed to be similar to that of teicoplanin.

Oritavancin, however, has additional mechanisms of action. The hydrophobic 4'-chlorobiphenylmethyl side chain on the disaccharide sugar of oritavancin facilitates direct interaction with the bacterial cell membrane, providing additional stability during oritavancin's interaction with lipid II (Allen and Nicas 2003). Oritavancin can also bind to D-alanyl-D-lactate, and this affinity is enhanced by its ability to form noncovalent dimers before attachment to the bacterial peptidoglycan cell wall. This dimerization is possible because of interactions between the disaccharides attached to residue 4, the chlorine on ring 2, and the 4-epi-vancosamine on ring 6, also found with the underivatized natural product chloroeremomycin (A28246B), which already shows superior dimerization to vancomycin (Linsdell et al. 1996; Allen et al. 2002). In addition to the above, oritavancin has been reported to inhibit transpeptidation. This action is attributed to the 4'-chlorobiphenylmethyl side chain, which allows binding of the drug to the pentaglycyl bridge, a secondary site in peptidoglycan, and likely contributes to oritavancin's activity against vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *Enterococcus* isolates (Leimkuhler et al. 2005; Mitra et al. 2015). The 4'-chlorobiphenylmethyl side chain of the molecule is also credited for Gram-positive bacterial cell death via membrane depolarization and increased mem-

brane permeability. This mechanism, which leads to disruption of membrane ultrastructure, has been shown in vitro to effect both antibacterial activity against stationary-phase inocula of staphylococci and rapid killing of susceptible Gram-positive pathogens. Interestingly, oritavancin additionally shows activity against *S. aureus* biofilms in vitro (Belley et al. 2009). Finally, oritavancin may also have some effect on inhibition of RNA synthesis but a direct effect is questionable (Allen and Nicas 2003).

Telavancin differs from vancomycin by having a dual mechanism of action, thought to be responsible for its rapid, dose-dependent bactericidal activity (Higgins et al. 2005). Like vancomycin and the other members of this class, telavancin has a glycopeptide core that binds with high affinity to the D-alanyl-D-alanine terminus of cell-wall precursors through a network of hydrogen bonds and hydrophobic packing interactions (Karlowsky et al. 2015). Thus, telavancin inhibits synthesis of peptidoglycan by binding to late-stage precursors. This binding prevents both polymerization of the lipid II precursor and subsequent cross-linking (Lunde et al. 2009). In contrast to vancomycin, telavancin does not inhibit autolysis (JL Pace, unpubl.). However, like oritavancin, telavancin does bind to bacterial cell membranes and cause a rapid, concentration-dependent depolarization of the plasma membrane, increased permeability, and leakage of cellular adenosine triphosphate and potassium (Higgins et al. 2005). The timing of these membrane changes correlates with the rapid, concentration-dependent bacterial killing seen with this agent, suggesting that telavancin's bactericidal activity results from the dissipation of cell membrane potential and an increase in membrane permeability (Higgins et al. 2005). Telavancin is also 10 times more active than vancomycin in inhibiting both the transglycosylation and the synthesis of peptidoglycan because of its ability to bind to bacterial cell membranes (Debabov et al. 2002; Higgins et al. 2005; Lunde et al. 2009). An alternative mechanism has been proposed for lipoglycopeptides suggesting that the liposaccharide elements of lipoglycopeptides interact directly with and inhibit transglycosylase enzymes that

mediate the polymerization of precursors into immature, un-cross-linked peptidoglycan (Ge et al. 1999; Goldman et al. 2000; Kerns et al. 2002; Chen et al. 2003); however, in the case of telavancin, this mechanism is not supported by observations that inhibition of transglycosylase is completely antagonized by the cell-wall-mimicking tripeptide dKAA (Higgins et al. 2005). Finally, it is hypothesized that telavancin inhibits bacterial lipid synthesis (Debabov et al. 2002).

MECHANISMS OF RESISTANCE

Gram-negative bacilli are intrinsically resistant to vancomycin and lipoglycopeptides, because the presence of the outer membrane prevents these large, complex molecules from entering the cell and binding to their target sites. In Gram-positive bacteria, the onset of vancomycin resistance was long delayed compared with that for other nonglycopeptide antibiotics. The first vancomycin-resistant clinically isolated strain was reported in 1987, more than 30 years after the introduction of vancomycin (Leclercq et al. 1988). Today more than 30% of intensive care unit (ICU) *Enterococcus faecalis* infections are vancomycin-resistant *Enterococcus* (VRE) and, following the emergence of vancomycin resistance in *Enterococcus*, rapid resistance developed in *S. aureus* (VRSA) as a result of horizontal gene transfer from resistant enterococci (Nebreda et al. 2007; Werner et al. 2008; Zhu et al. 2010; Fernandes et al. 2015). The first fully VRSA strain (vancomycin, minimum inhibitory concentration [MIC] > 256 µg/mL; teicoplanin, MIC = 128 µg/mL) was reported in 2002 (Howden et al. 2010) with an increasing number of cases each year in the United States (Walters et al. 2015).

Because vancomycin forms complexes with peptidoglycan precursors, its activity is determined by its binding affinity to precursor substrates. Indeed, the main mechanism of resistance to vancomycin is owing to the presence of enzymes that produce lower-affinity binding precursors in which the carboxy-terminal D-alanine residue is replaced by either D-lactate, resulting in a 1000-fold decrease in binding affinity

caused by the loss of a hydrogen bond interaction (Pootoolal et al. 2002), or D-serine, resulting in a sixfold loss of binding affinity likely caused by steric hindrance. There are six different molecular resistance elements that confer resistance to vancomycin by altering the structure of the peptidoglycan precursors (Table 3) (Cetinkaya et al. 2000; Courvalin 2006).

Van-A type resistance is the most frequently encountered mechanism of resistance in enterococci and is the only one detected in *S. aureus* to date (Perichon and Courvalin 2009). Transposon Tn1546 and closely related elements encoding nine polypeptides responsible for vancomycin resistance mediate this by changing the carboxyl terminus peptidoglycan precursor from D-alanine to D-lactate. These proteins include those needed for the synthesis of modified peptidoglycan precursors (VanH and VanA), hydrolysis of normal precursors (VanX and VanY), regulation of expression of resistance (VanR and VanS), transposition (products of ORF1 and ORF2), and an unknown function (VanZ). VanH is a dehydrogenase that reduces pyruvate to D-Lac, and VanA is a ligase allowing the formation of a D-Ala-D-Lac depsipeptide that replaces the D-Ala-D-Ala dipeptide. Furthermore, indigenous concentrations of D-Ala-D-Ala are reduced by VanX and VanY. VanX is a D,D-dipeptidase that hydrolyzes the D-Ala-D-Ala formed by the host chromosomal D-Ala-D-Ala ligase, and VanY is a D,D-carboxypeptidase that hydrolyzes the terminal D-Ala of pentapeptide precursors that are produced if elimination of the D-Ala-D-Ala is not complete (Arthur et al. 1996). Inducible expression of this resistance is regulated by the VanR/VanS two-component system. VanS is the membrane-associated sensor that is activated by the presence of glycopeptides and transfers a phosphoryl group to VanR, which is the response regulator that activates the cotranscription of the *vanH*, *vanA*, *vanX*, and *vanY* genes by binding to the P_{RES} promoter (Arthur et al. 1992), and of the *vanR* and *vanS* genes by binding to the P_{REG} promoter (Depardieu et al. 2007). This transposition confers high-level resistance to vancomycin and teicoplanin as well as telavancin, although telavancin

Table 3. Glycopeptide and lipoglycopeptide antibacterial resistance determinants (Courvalin 2006)

Resistance type	Resistance element	Expression	Location	Modified target	MIC (μg/mL)			
					Vancomycin	Teicoplanin	Oritavancin	Telavancin
Van-A	Tn1546	Inducible	Plasmid	D-alanine D-lactate	64–1000	16–512	8–16 ^a	32 ^a
Van-B	Tn1547 or Tn1549	Inducible	Plasmid	D-alanine D-lactate	4–1000	0.5–1	8–16 ^a	
Van-C		Constitutive Inducible	Chromosome	D-alanine D-serine	2–32	0.5–1	8–16 ^a	
Van-D		Constitutive	Chromosome	D-alanine D-lactate	64–128	4–64		
Van-E		Inducible	Chromosome	D-alanine D-serine	8–32	0.5		
Van-G		Inducible	Chromosome	D-alanine D-serine	16	0.5		

^aSelected in resistance passaging studies.

MICs remain 32- and 128-fold lower compared with those of vancomycin and teicoplanin (Karlowsky et al. 2015). The VanA-type resistance is transmittable from *Enterococcus* species to *S. aureus* and is the main resistance mechanism for VRSA (Perichon and Courvalin 2009).

Like VanA, VanB-type resistance is caused by the presence of a transposition (Tn1547 or Tn1549), resulting in the altered synthesis of the depsipeptide D-Ala-D-Lac to replace the dipeptide D-Ala-D-Ala and leading to decreased lipid II binding by the antimicrobials. Also, the organization and functionality of the vanB cluster are similar to that of vanA, encoding a dehydrogenase, ligase, and a dipeptidase, all of which have a high level of sequence identity (67%–76%) to the corresponding proteins of the vanA operon. However, the regulatory proteins in VanB type resistance only distantly relate to VanRS (34% and 24% identity) and are only induced by vancomycin but not teicoplanin; hence, strains with this phenotype have high-level resistance only to vancomycin (Courvalin 2006).

Likewise, VanD-type resistance also results in the production of peptidoglycan precursors ending in D-Ala-D-Lac. The organization of the vanD operon is similar to that of vanA and vanB; however, vanD is located exclusively on the bacterial chromosome and resistance is not transferable by conjugation to other enterococci (Depardieu et al. 2004). In addition, VanD-type strains do not have D,D-dipeptidase activity, which is not needed because various point mutations also result in null D-Ala-D-Ala ligase activity (Arthur et al. 1998; Depardieu et al. 2003b). Interestingly, because of this defect in indigenous D-Ala-D-Ala production, VanD-type strains require constitutive expression of these resistance elements to survive and achieve this via mutations in the VanS_D sensor or VanR_D regulator (Depardieu et al. 2003b, 2004).

The VanC-type resistance is found in *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens* and results in production of peptidoglycan precursors terminating in D-Ala-D-Ser (Reynolds and Courvalin 2005). This cluster contains three main genes that are needed to confer resistance: VanT is a

membrane-bound serine racemase that produces D-Ser; VanC is a ligase that catalyzes synthesis of D-Ala-D-Ser; and VanXY_C possesses both D,D-dipeptidase and D,D-carboxypeptidase activities and hydrolyzes precursors ending in D-Ala-D-Ala. The VanC phenotype is expressed constitutively or inducibly on the chromosome and confers resistance to low levels of vancomycin but not to other glycopeptides (Reynolds and Courvalin 2005).

VanE-type resistance is organized similarly to VanC and is found in intrinsically resistant *E. faecalis*. Unlike VanC, the VanE phenotype is only inducibly expressed (Courvalin 2006).

The VanG-type resistance consists of genes from various van operons: VanG is a D-Ala-D-Ser ligase (similar to VanC-type strains); VanXY_G is a putative bifunctional D,D-peptidase and D,D-carboxypeptidase; VanT_G is a serine racemase (similar to VanE-type strains); and VanR_G and VanS_G make up the two component regulatory response proteins (highest similarity to the VanD-type strains). Like VanC and VanE phenotypes, the VanG-type strains produce D-Ala-D-Ser and only have intermediate-level resistance to vancomycin (Depardieu et al. 2003a).

VISA strains, unlike those described above, do not carry imported foreign genetic elements; rather, resistance occurs owing to mutations that appear in the invading pathogen during treatment. Although the MIC value of these strains are modest (MIC = 2–16 µg/mL), vancomycin treatment of VISA infections often ends in treatment failure (Linares 2001; Fridkin et al. 2003). Most commonly, these strains have emerged in patients with MRSA infections undergoing prolonged vancomycin therapy (Linares 2001; Chen et al. 2003; Howden et al. 2010). Although VISA isolates have differing point mutations, they do share common abnormal properties including increased cell-wall material, an aberrant separation of daughter cells at the end of cell division, and altered (usually decreased) rates of autolysis (Hiramatsu 2001; Walsh and Howe 2002; Sieradzki and Tomasz 2003; Pfeltz and Wilkinson 2004; Gardete et al. 2012; Howden et al. 2014; Karlowsky et al. 2015). The most frequent genetic changes in VISA strains are in *walkR* and *vraSR* (two-

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component sensory regulatory system controlling the transcription of genes in cell-wall synthesis), and *rpoB* (RNA polymerase) genes (Howden et al. 2008, 2011; Kato et al. 2010; Watanabe et al. 2011; Hafer et al. 2012). It is hypothesized that these point mutations decrease the cross-linking of peptidoglycan strands, which results in an increase in the number of free D-Ala-D-Ala residues in the cell wall that bind and sequester vancomycin (Sieradzki et al. 1999; Hiramatsu 2001; Sieradzki and Tomasz 2003). These D-Ala-D-Ala residues can be maintained in the mature peptidoglycan because of low carboxypeptidase activity, and vancomycin-resistant laboratory mutants have shown an increased capacity to bind vancomycin in their cell walls (Sieradzki and Tomasz 1997). Not only does this result in “decoy” targets, but binding of the large glycopeptide molecules would further hinder the progress of the antibiotic through the mesh-like cell wall to its lethal target, the lipid-linked peptidoglycan precursor, thus creating a “drug capture” (Perichon and Courvalin 2009) and “clogging” phenomenon (Sieradzki et al. 1999).

Reported in vitro mutational frequency of resistance against dalbavancin, oritavancin, and telavancin has been remarkably, although not unexpectedly, limited (Arthur et al. 1999; Krause et al. 2005; Sahm et al. 2006; Goldstein et al. 2007; Laohavaleeson et al. 2007; Kosowska-Shick et al. 2009; Song et al. 2013). Resistance to vancomycin required ~30 years in the clinic to become prevalent and required concomitant acquisition of several exogenous genes. With the semisynthetic lipoglycopeptide antimicrobials showing additional mechanisms of action, resistance frequency is expected to be lower.

Like teicoplanin, telavancin retains activity against strains that express vanB, but not vanA as it induces expression of the latter (Zhanel et al. 2010). In a study by Kosowska-Shick and colleagues that serially passaged 10 strains of MRSA to evaluate the potential for emergence of resistance, spontaneous mutants resistant to telavancin were detected in only 1 of the 10 MRSA strains tested. The MIC for that strain increased from 0.25 to 2 µg/mL after 43 days of serial passing; no further increase in the

MIC of this mutant occurred when serial passaging was continued to a maximum of 50 days (Kosowska-Shick et al. 2009). In another study, six species of Gram-positive bacteria, including *S. aureus*, vancomycin-resistant enterococci, and multidrug-resistant *Streptococcus pneumoniae*, were exposed to sub-MIC concentrations of telavancin for 10 days to attempt to isolate resistant mutants. However, this failed to produce any organisms that were resistant to telavancin; mutation frequencies were $<1 \times 10^{-9}$ for all isolates tested (Sahm et al. 2006). Laohavaleeson et al. (2007) also reported that telavancin showed a low frequency of spontaneous resistance among staphylococci and enterococci, with low spontaneous resistance rates for *S. aureus* strains and both VanA *E. faecalis* and *Enterococcus faecium* ($<1 \times 10^{-9}$ and $<1 \times 10^{-7}$, respectively). Similarly, Krause et al. (2005) reported telavancin to have a low potential for resistant-mutant selection among *S. aureus* (MSSA, MRSA) and vancomycin-susceptible enterococci because it failed to select mutants with MICs >4 times those of parental strains following 20 passages. Two mutants with telavancin MICs 4 times that of the parental strain (32 µg/mL) were selected from vancomycin-resistant enterococci strains (VanA) used; mutant phenotypes were stable on extended subculture, and no changes were observed in growth properties of isolates with reduced susceptibility to telavancin. Song et al. (2013) sought to determine mechanisms of decreased telavancin susceptibility in a laboratory-derived mutant of *S. aureus*, TlvDSMED1952, and showed extensive changes in the mutant transcriptome compared with the susceptible parent strain, MED1951. Up-regulated genes in TlvDSMED1952 included cofactor biosynthesis genes, cell-wall-related genes, fatty acid biosynthesis genes, and stress genes, whereas down-regulated genes included lysine operon biosynthesis genes and *lrgB* (induced by telavancin in susceptible strains), *agr* and *kdpDE* genes, various cell surface protein genes, phenol-soluble modulins, several protease genes, and genes involved in anaerobic metabolism. The investigators reported that TlvDSMED1952 showed various features simi-

lar to VISA and strains with decreased daptomycin susceptibility, but also showed its own unique features. Consistent with these findings was that in vitro resistance was more frequently selected in VanA *E. faecalis*, and that an increased IC₅₀ for [¹⁴C]-acetate incorporation into both cellular phospholipids and saponifiable fatty acids appeared to correlate with the increased MICs, whereas peptidoglycan synthesis inhibition was unchanged (Debabov et al. 2002; Higgins et al. 2005; JL Pace, unpubl.). Heteroresistance has not been detected in isolates of *S. aureus*, nor has resistance been detected in isolates of *S. aureus* or *S. pneumoniae* collected either through clinical trials or through global surveillance (Kosowska-Shick et al. 2009; Krause et al. 2012). MIC₉₀ values of 0.25–0.5 µg/mL (*S. aureus* isolates including MRSA) and 0.03 µg/mL (*S. pneumoniae*) have been reported (Theravance 2014).

Similar to teicoplanin and telavancin, dalbavancin resistance is observed for strains that express the *vanA* operon, but not *vanB*, as this agent only induces expression of the former. Prospective worldwide surveillance from 2002 to 2012 of the in vitro potency of dalbavancin against >150,000 Gram-positive isolates found no evidence of glycopeptide-resistant or glycopeptide intermediate *S. aureus* by CLSI criteria (MIC₉₀ was 0.06 µg/mL for *S. aureus*). Isolates from phase-III clinical trials showed similar susceptibility profiles to those detailed in the surveillance studies (Jones et al. 2013a,b). The potential for staphylococci to develop resistance to dalbavancin was studied by Goldstein et al. (2007) by means of direct selection and by serial passage at subtherapeutic concentrations. In direct selection experiments with the six strains tested (one MSSA strain, three MRSA strains, one VISA strain, and one methicillin-resistant *S. epidermidis* strain), no colonies with increased MICs were obtained on dalbavancin-containing plates (frequency <10¹⁰). The same six strains were passaged in the presence of sub-inhibitory concentrations of dalbavancin. After 20 passages, four strains (including the VISA strain) had a dalbavancin MIC that was within one doubling dilution of the MIC obtained before the 20 passages. For two MRSA strains,

increases of two or three dilutions were seen (to 0.25 and 0.5 µg/mL). When these isolates were grown on drug-free medium for 3 consecutive days and retested, the MICs were equivalent or within one doubling dilution of the starting MICs.

Oritavancin resistance among clinical isolates has not been detected so far (Allen and Nicas 2003). However, a moderate level of resistance (MIC ≤ 16 µg/mL) to the drug has been observed in the laboratory among *Enterococcus* isolates showing the VanA and VanB phenotypes and can occur by various mechanisms (Arthur et al. 1999). Total replacement of the peptidoglycan precursors terminating in D-alanine by isolates capable of producing peptidoglycan precursors terminating in D-lactate can confer resistance to oritavancin. This can be achieved in vitro by either increasing resistance gene expression or reducing production of D-Ala-D-Ala. Resistance to the drug may also occur with expression of the *vanZ* gene. Additionally, mutations in the *vanSB* sensor gene of the *vanB* cluster confer cross-resistance to teicoplanin and oritavancin (Arthur et al. 1999), making it is quite likely that emergence of resistance to oritavancin may occur with widespread clinical use.

CONCLUDING REMARKS

Over the past 60 years, vancomycin has come to play a critical, lifesaving role in a physician's armamentarium against serious Gram-positive bacterial infections. This antibiotic's remarkable longevity of use and delayed onset of resistance speaks to the unique mode of action of the glycopeptide antibiotic class. Since 2011, three semisynthetic lipoglycopeptide antibacterials have gained FDA approval in the United States. The development of these new agents was guided by the extensive body of earlier research on vancomycin—its mechanism of action, mechanisms of resistance, and therapeutic applications. These targeted efforts have imparted new, beneficial properties to the glycopeptide class. One can only hope that, through stewardship and appropriate practice, the successful clinical use of vancomycin and the newer gly-

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copeptide agents will continue far into the future, to the benefit of all.

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