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Current Perspectives on Glycopeptide Resistance

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INTRODUCTION

The glycopeptide antibiotics, vancomycin and teicoplanin, inhibit cell wall synthesis in gram-positive bacteria by interacting with the terminal D-alanyl–D-alanine (D-Ala–D-Ala) group of the pentapeptide side chains of peptidoglycan precursors. This interaction prevents the transglycosylation and transpeptidation reactions required for polymerization of peptidoglycan. Glycopeptide molecules are large (vancomycin and teicoplanin have molecular weights of 1,448 and approximately

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1,900, respectively) and so cannot permeate the gram-negative outer membrane to reach this target site. Thus, most gram-negative bacteria are resistant to their action, while nearly all gram-positive bacteria are susceptible.

Since the introduction of vancomycin in the 1950s (161), the glycopeptide group of antimicrobial agents has been used to treat disease caused by gram-positive bacteria showing either intrinsic (as in *Clostridium difficile* or *Corynebacterium jeikeium*) or acquired (as in methicillin-resistant *Staphylococcus aureus* [MRSA] or β-lactam-resistant *Streptococcus pneumoniae*) resistance to other agents. Glycopeptides are also used to treat disease in patients with intolerance to favored antibiotics, particularly β-lactams. Vancomycin is relatively inconvenient to administer, requiring slow intravenous infusion. Untoward effects (354) include "red man" syndrome during or after administration and also nephrotoxicity and ototoxicity, particularly in association with aminoglycoside administration. Glycopeptides are relatively expensive antibiotics, and there are fears that their use may encourage the emergence of resistance. For all of these reasons, the use of glycopeptides today is still limited to situations that suggest resistance or allergy to other antibiotics (405).

The introduction of new antibiotics into clinical use is usually followed by the fairly rapid emergence of bacterial isolates resistant to them. In this respect, vancomycin was somewhat atypical. For almost 30 years following its introduction, resistance to vancomycin was reported only rarely and appeared to have little clinical significance. However, in 1988 there were two reports of plasmid-mediated, high-level resistance to both vancomycin and teicoplanin in *Enterococcus* spp. (230, 383), a genus associated with resistance or tolerance to many classes of antibiotics. These and subsequent reports alerted microbiologists to the possibility of glycopeptide resistance in enterococci, and many laboratories began screening for it. It became apparent that not all glycopeptide-resistant enterococci displayed the same type of resistance. Most isolates in these early reports showed high-level resistance to vancomycin (MIC, >128 μg/ml) and cross-resistance to teicoplanin (MIC, ≥8 μg/ml). However, other isolates showed low-level resistance to vancomycin alone (MICs, ≤64 μg/ml) (326, 409, 414). The increased interest in and awareness of glycopeptide resistance also resulted in its recognition as an intrinsic characteristic of some occasional human pathogens such as lactobacilli, leuconostocs, pediococci, and *Erysipelothrix rhusiopathiae*. In addition, coagulase-negative staphylococci (CNS) with low-level glycopeptide resistance were detected.

Glycopeptide resistance was first reviewed in this journal in 1990 (203). Since then, the problems posed by resistant organisms, particularly enterococci, have become increasingly widespread. In the last 5 years, our understanding of the genetics and biochemical basis of resistance has increased greatly. Many centers have now reported either sporadic isolates or clusters of infections and colonizations caused by glycopeptide-resistant enterococci. The development of molecular typing methods for these organisms has allowed the epidemiology of clusters to be investigated. Other areas of key importance are the detection of glycopeptide-resistant organisms in the clinical laboratory and options available for the management of infected patients. The aim of the present article is to provide an update on these aspects of glycopeptide resistance.

GENETICS AND BIOCHEMICAL BASIS OF GLYCOPEPTIDE RESISTANCE

Enterococci

Preliminary studies. Early data provided no evidence for enzymatic inactivation of glycopeptide antibiotics by resistant bacteria (203). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis studies demonstrated the appearance of new proteins in cell membrane preparations derived from isolates of *Enterococcus faecalis* or *E. faecium* with either high- or low-level vancomycin resistance (203). The proteins produced by high- and low-level resistant enterococci had slightly different sizes (39 and 39.5 kDa, respectively) (287, 345, 409, 414) and showed no antigenic cross-reactivity (7). However, despite these differences, both proteins were produced only after exposure of the organisms to vancomycin. This observation was consistent with the growth kinetics of broth-grown, high- or low-level resistant isolates which showed that, after exposure to vancomycin, there was a lag phase of several hours before normal growth resumed (287, 345, 409). In addition, antisera raised against the 39- or 39.5-kDa proteins did not react with membrane preparations from nonexposed cells (7). These data suggested that vancomycin resistance was inducible and that exposure to vancomycin caused resistance genes to be expressed, with the subsequent production of novel proteins.

Despite evidence that the 39- and 39.5-kDa proteins were structurally distinct, experimental data suggested that actual mechanisms of high- and low-level resistance were similar. Investigation of *E. faecalis* A256 (exhibiting high-level resistance) and *E. faecium* D366 (exhibiting low-level resistance) showed that fractions which made up cell wall material and some cytoplasmic membrane proteins were able to bind vancomycin and teicoplanin when prepared from uninduced cells of these two isolates. Negligible binding occurred when similar fractions were prepared from induced cells. However, removal of proteins from induced preparations, by boiling with SDS, restored the ability to bind glycopeptides (6). These inducible, membrane-associated proteins could also protect exogenous pentapeptide from glycopeptides. It had previously been suggested that the 39- and 39.5-kDa proteins might bind to and mask the D-Ala–D-Ala target site of glycopeptides (224). However, al-Obeid and colleagues found that protection of exogenous pentapeptide by induced proteins was a time-dependent, heat-labile process, suggesting enzymatic modification rather than simple binding (6).

Although the 39- and 39.5-kDa proteins are the most apparent new species present in induced cells, other minor protein differences have also been reported (7, 414). This suggested that the mechanism of glycopeptide resistance might be complex and not the result of production of a single protein.

Genetic basis of VanA resistance. A major advance towards elucidating the mechanism of high-level glycopeptide resistance involved cloning the gene that encodes the 39-kDa protein. In *E. faecium* BM4147, this gene is located on a 34-kb nonconjugative plasmid designated pIP816 (54, 99, 230). A cloned 1,761-bp fragment of this plasmid encoded production of a single peptide which, when expressed in *Escherichia coli*, had an electrophoretic mobility identical to that of the 39-kDa resistance-associated protein from the parent *E. faecium* (99). Sequencing the fragment revealed a single 1,029-bp open reading frame (ORF), designated *vanA*. The amino acid sequence of the predicted VanA peptide was consistent with the experimentally determined N-terminal sequence of the 39-kDa protein from *E. faecium* BM4147 (99). The VanA peptide is structurally similar to the D-Ala–D-Ala ligase enzymes from *E. coli* and *Salmonella typhimurium* (99). These enzymes are responsible for production of the D-Ala–D-Ala dipeptide, which in gram-positive bacteria is the target for glycopeptide antibiotics. The VanA peptide allowed growth at the nonpermissive temperature of an *E. coli* isolate with a temperature-sensitive D-Ala–D-Ala ligase, supporting its biological activity (99). Subsequently, the purified VanA peptide was shown to possess

D-Ala–D-Ala ligase activity, although it had a wider substrate specificity than comparable enzymes from gram-negative bacteria (58). These data suggested that the VanA peptide served to produce a D-Ala–X component for incorporation into peptidoglycan precursors which would then show reduced binding of glycopeptides.

Although the *vanA* gene encodes a D-Ala–X ligase, it is not sufficient to confer glycopeptide resistance on susceptible enterococci. A 5.5-kb *Bgl*II-*Xba*I fragment of pIP816 was necessary for expression of inducible, high-level resistance (16). Cloning of this fragment, to produce a recombinant plasmid designated pAT80 (16), allowed sequencing of DNA upstream of *vanA*. This revealed another ORF of 983 bp, designated *vanH*, which overlaps *vanA* by 5 bp (19). The predicted VanH product appeared similar to 2-hydroxycarboxylic acid dehydrogenase, and it was postulated that it might synthesize the substrate used by the VanA ligase (19). Purification of VanH confirmed such a metabolic linkage. The VanH peptide reduced 2-keto acids to produce D-hydroxy acids (59). The VanA ligase then catalyzed synthesis of depsipeptides by formation of an ester bond between D-Ala and these products (59). The subsequent incorporation of these depsipeptides into peptidoglycan precursors could be catalyzed by purified *E. coli* D-Ala–D-Ala adding enzyme or by crude extracts prepared from either glycopeptide-resistant *E. faecium* BM4147 or a susceptible derivative of BM4147 which lacked resistance plasmid pIP816 (59). This indicates that incorporation of the depsipeptide into peptidoglycan precursors is undertaken by chromosomally encoded proteins and does not require further products encoded by the glycopeptide resistance plasmid (59).

The identity of the D-hydroxy acid produced by the VanH peptide and used as a substrate by VanA was elucidated by insertional inactivation of the *vanH* gene (15). Such inactivation reduced the vancomycin MIC for an *E. faecalis* JH2-2 derivative carrying plasmid pAT80 from 1,024 to 1 μg/ml. However, resistance could be restored by the addition of either DL-2-hydroxybutyrate or D-lactate to the growth medium. As D-lactate could restore full resistance at lower concentrations than DL-2-hydroxybutyrate, it was suggested that D-lactate was produced by VanH from pyruvate and was then incorporated into the peptidoglycan precursors (15). Direct analysis of the peptidoglycan precursors of clinical isolates or laboratory-derived, glycopeptide-resistant and -susceptible enterococci confirmed that resistant cells did produce new precursors (4, 44, 179, 253). The precursors of induced, resistant cells contained the amino acids alanine, glutamic acid, and lysine in a ratio of 2:1:1, rather than in the 3:1:1 ratio found in precursors of susceptible cells (4, 253). Mass spectrometry suggested that the precursors of resistant cells also contained D-lactate (4, 179, 253). This was confirmed by the release of D-lactate following treatment of precursors of resistant cells with a DD-peptidase (253).

Further sequencing of DNA downstream from *vanH* and *vanA* on plasmid pAT80 identified a third ORF, designated *vanX*, which was separated from *vanA* by only 8 bp (16). Although the predicted VanX peptide showed no amino acid homology with known peptides, insertional inactivation of the *vanX* gene demonstrated that it was essential for glycopeptide resistance (16). An *E. faecalis* JH2-2 derivative carrying pAT80 produced peptidoglycan precursors terminating in D-Ala–D-lactate and D-Ala–D-Ala in the ratio of 49:1. However, after inactivation of *vanX*, this ratio decreased to 1:1 (313) and glycopeptide resistance was lost (16). The *vanX* gene has recently been sequenced, and the VanX peptide was identified as a DD-dipeptidase, as demonstrated by its ability to hydrolyze D-Ala–D-Ala. It did not hydrolyze D-Ala–D-lactate, pentapeptide, or pentadepsipeptide (313). The predicted amino acid sequence of the VanX peptide has no hydrophobic domains, suggesting that it is not membrane associated, and indeed, most of the DD-peptidase activity was located in cytoplasmic cell fractions (313). Previously, Billot-Klein and colleagues had noted a reduction in the concentration of D-Ala–D-Ala present in the cytoplasm of induced, resistant cells (44). As no tetrapeptide precursors were detected in this study, they had suggested the existence of a cytoplasmic enzyme that could cleave D-Ala–D-Ala but which did not act upon pentapeptide precursors (44). These suggestions are consistent with the characteristics of VanX. It had also been postulated that synthesis from chromosomal genes of glycopeptide-susceptible peptidoglycan precursors containing the D-Ala–D-Ala dipeptide would be down-regulated in glycopeptide-induced resistant cells (59). However, current data suggest that this is not the case. It now appears that VanX is the major component responsible for preventing synthesis of peptidoglycan precursors containing this dipeptide (313). Functions have now been assigned to the VanH, VanA, and VanX peptides, which are, with the exception of regulatory gene products (see below), the only essential requirements for glycopeptide resistance. Therefore, actual incorporation of the D-Ala–D-lactate depsipeptide must involve chromosomally encoded enzymes, as inferred previously (59).

Regulation of VanA resistance. The inducible nature of glycopeptide resistance in most VanA enterococci suggests that expression is regulated at the genetic level. Initial experimental evidence for this was provided by Brisson-Noël and colleagues (54). A recombinant plasmid carrying a 4-kb *Eco*RI fragment of plasmid pIP816 conferred vancomycin resistance on *E. faecalis* JH2-2 (MIC, 128 μg/ml). The presence of an additional 6-kb *Eco*RI fragment, located upstream from the first and which itself did not confer resistance, increased the level of vancomycin resistance dramatically (MIC, 4,000 μg/ml) (54). Subsequently, it was found that the 5.5-kb *Bgl*II-*Xba*I fragment carried by pAT80 (which carries intact *vanH*, *vanA*, and *vanX* genes) was sufficient to confer inducible, high-level vancomycin resistance in *E. faecalis* JH2-2 (16). Sequencing of the DNA upstream from the *vanH* translation initiation codon revealed two other ORFs that have been designated *vanS* and *vanR* (16). In a separate study, a gene identical to *vanR* was sequenced from the VanA resistance plasmid pHKK100 (175).

Analysis of the predicted amino acid sequences of the *vanS* and *vanR* gene products indicated similarity with two-component signal-transducing regulatory systems that sense and respond to environmental stimuli (12, 16, 329, 423). These systems usually consist of a transmembrane sensory peptide (a histidine protein kinase) that contains a specific histidine residue which is phosphorylated in response to specific environmental stimuli. The phosphoryl group is then passed to an aspartate residue of a second peptide, the response regulator. This response regulator peptide has a DNA binding domain, and once phosphorylated, the peptide affects transcription of specific genes (329).

The predicted *vanS* gene product appeared related to histidine protein kinases, and its primary sequence revealed two clusters of hydrophobic amino acids, consistent with two membrane-spanning domains (16). In addition, it has been proposed that the VanS peptide also consists of a cytosolic kinase domain and an extracellular sensory domain (423). The *vanR* product, which shows similarity to response regulator peptides, has been purified, and the cytosolic domain of the *vanS* product has been obtained as a fusion product with a maltose-binding protein (423). This study confirmed the postulated biological activity of the two peptides. The cytosolic domain of

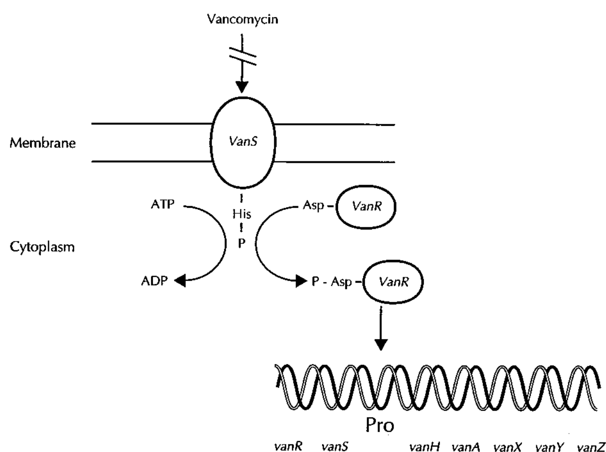

FIG. 1. Regulation of the *van* gene cluster of *E. faecium* BM4147 (*vanA* genotype). The presence of vancomycin (or teicoplanin) indirectly causes autophosphorylation of a histidine residue on the sensory peptide, VanS. The phosphoryl group is transferred to an aspartate residue on the VanR response regulator. Phospho-VanR then acts on a promoter (Pro), allowing transcription of the *van* cluster and expression of glycopeptide resistance.

VanS does indeed have kinase activity and is able to autophosphorylate in the presence of ATP. The resulting phospho-VanS was able to transfer the phosphoryl group rapidly to purified VanR (423) (Fig. 1).

The role of these two peptides in the regulation of VanA glycopeptide resistance has been confirmed by insertional inactivation of the genes. Inactivation of the *vanS* gene had no effect on the level of resistance shown by a laboratory-derived strain, although the actual level of transcription of resistance genes decreased (16). In contrast, inactivation of *vanR* caused a vancomycin-resistant laboratory-derived strain (16) and a clinical isolate (175) to become susceptible to glycopeptides. These data support the role of VanR as a transcriptional activator and suggest that it is stimulated by VanS (16). The phosphorylated VanR peptide acts on a promoter that lies between *vanS* and *vanH* and from which the *vanH*, *vanA*, and *vanX* genes are cotranscribed (16). Phospho-VanR is relatively stable, which might assist prolonged expression of glycopeptide resistance genes (423).

Continued expression of glycopeptide resistance in a strain with an inactivated *vanS* gene (16) suggests that interactions are possible between VanR and the histidine protein kinase of another two-component regulatory system(s), although at present there is no experimental evidence to suggest that VanR may be phosphorylated by peptides other than VanS (16, 423). By implication, this other regulatory system(s) is likely to be located on the bacterial chromosome. There is precedent for *trans*-interaction between chromosomally encoded regulatory genes and plasmid-encoded glycopeptide resistance genes. Arthur and colleagues demonstrated that plasmid pAT87, carrying just the *vanH*, *vanA*, and *vanX* genes, was not able to confer glycopeptide resistance in susceptible strains of *E. faecalis*. However, chromosomal integration of a second recombinant plasmid (pAT89) carrying the *vanS* and *vanR* genes allowed resistance to be expressed (16).

The environmental stimulus that triggers the initial phosphorylation of VanS has not been identified but is probably related to the presence of vancomycin and its interaction with the D-Ala–D-Ala target site (12), which inhibits transglycosylation. As supportive evidence, moenomycin, which acts as a competitive inhibitor of transglycosylases, was the only non-glycopeptide antibiotic able to induce glycopeptide resistance in a VanA strain of *E. faecium* (177).

Identification of VanA resistance transposon Tn*1546*. As described above, resistance plasmid pIP816 carried a five-gene *van* cluster necessary for glycopeptide resistance (*vanR*, *vanS*, *vanH*, *vanA*, and *vanX*). A DNA probe specific for the *vanA* gene hybridized with heterogeneous plasmids from clinical isolates and transconjugants that displayed high-level resistance to vancomycin and teicoplanin (98). The presence of *vanA*-like genes on these plasmids implied that the entire *van* cluster would also be present. The apparent dissemination of this gene cluster raised the possibility that it might be part of a transposable element (18). A transposon, designated Tn*1546*, was subsequently identified by sequencing the DNA flanking the *van* cluster of pIP816 (Fig. 2). This element is 10,851 bp in length and is bordered by imperfect, inverted 38-bp repeats (18). In addition to the five-gene *van* cluster, Tn*1546* also contains two ORFs, which are transcribed in opposite directions and which encode production of putative transposase and resolvase enzymes. There is considerable amino acid homology between these two predicted peptides and the transposases and resolvases of other transposons found in gram-positive bacteria. DNA hybridization and PCR studies have confirmed the presence of transposons related to Tn*1546* in highly glycopeptide-resistant enterococci other than *E. faecium* BM4147 (the host of pIP816) (18, 182). However, heterogeneity of the *van* cluster due to insertion of a novel DNA segment was observed during a recent study of VanA strains of *E. faecium* and *E. faecalis* isolated in the northeastern United States (182). It would therefore appear that the presence of the *van* cluster on a transposable element is a factor in the dissemination of *vanA*-mediated glycopeptide resistance among enterococci but that the detailed structure of these elements may vary.

Transposon Tn*1546* also carries two other ORFs, located downstream of the *van* cluster. These genes, which have been designated *vanY* and *vanZ*, are not required for the expression of glycopeptide resistance (17, 18). The *vanY* gene encodes a DD-carboxypeptidase which is not inhibited by penicillin (17, 424). This enzyme activity is associated with the cytoplasmic membrane and its production is inducible by vancomycin (17, 167, 424). This inducibility suggests that transcription of *vanY* may be regulated together with the *van* cluster. Insertional inactivation of *vanR* results in loss of DD-carboxypeptidase activity (174), which supports this possibility.

Prior to elucidation of the biochemical basis of glycopeptide resistance by production of altered peptidoglycan precursors, it had been proposed that DD-carboxypeptidase activity might be responsible. Excessive production of DD-carboxypeptidase would reduce the availability of pentapeptide precursors terminating in D-Ala–D-Ala and would prevent binding of glycopeptides (6). However, *vanY* is not located on pAT80, which confers high-level glycopeptide resistance (16), clearly demonstrating that it does not fulfill an essential function. The VanY peptide is able to remove either the terminal D-Ala or D-lactate group from peptidoglycan precursors, indicating that it also has DD-carboxyesterase activity (424).

Increasing the concentration of D-alanine in the growth medium from 0 to 25 mM reduced the vancomycin MIC for an *E. faecalis* JH2-2 derivative containing pAT80 from 2,048 to 16 μg/ml (14). However, if *vanY* was included on the recombinant plasmid, then no alteration in vancomycin resistance occurred over this range of D-alanine concentrations (14). High concentrations of D-alanine and other D-amino acids were previously shown to reduce the MICs for vancomycin-resistant enterococci (429). It has been proposed that the VanY peptide acts as a dispensable "failsafe," preventing incorporation of glycopep-

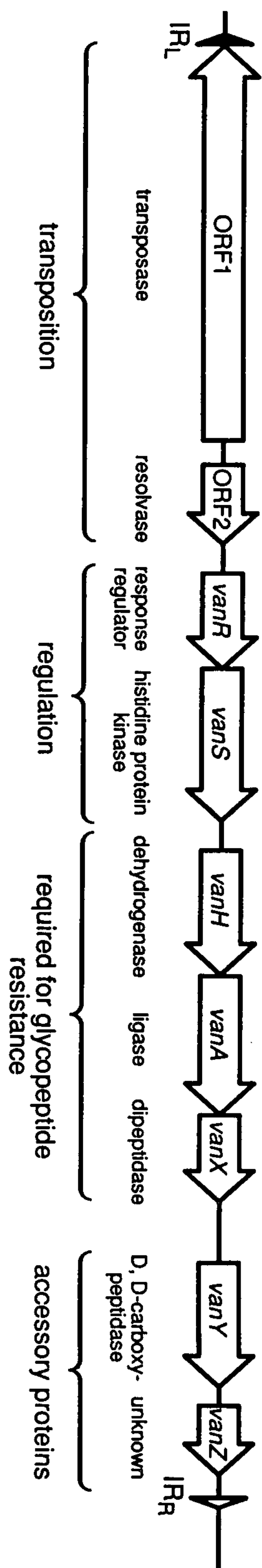

FIG. 2. Map of glycopeptide resistance transposon Tn*1546* from *E. faecium* BM4147. IR$_L$ and IR$_R$ indicate the left and right inverted repeats of the transposon, respectively. Adapted from reference 12 with the permission of the authors and publisher.

tide-susceptible precursors containing D-Ala–D-Ala into peptidoglycan (14). Under most conditions, this is brought about by efficient hydrolysis of the dipeptide by the VanX DD-peptidase. However, in the presence of excess D-Ala (or some other amino acids), some dipeptides are not hydrolyzed by VanX and are incorporated into precursors. In this situation, the VanY peptide cleaves the terminal D-Ala residue to produce tetrapeptide precursors that do not contain the target for glycopeptides.

In addition to the high-level glycopeptide resistance mediated by the *vanH*, *vanA*, *vanX*, and *vanY* genes, recent studies have indicated that Tn*1546* also encodes a second mechanism of glycopeptide resistance, mediated by the *vanZ* gene product (13). Introduction of a recombinant plasmid carrying only the *vanZ* gene increased the teicoplanin MIC for *E. faecalis* JH2-2 from 2 to 8 μg/ml but did not affect the MIC of vancomycin. Analysis of peptidoglycan precursors indicated that the resistant derivative continued to produce pentapeptide terminating in D-Ala–D-Ala (13). Insertional inactivation of *vanZ* restored teicoplanin susceptibility (13). The mechanism by which the VanZ peptide confers this low-level teicoplanin resistance has yet to be established.

VanB and VanC resistance phenotypes. By analogy with work undertaken on the VanA phenotype, the 39.5-kDa protein associated with low-level vancomycin resistance in *E. faecalis* and *E. faecium* and the undefined gene encoding its production were designated VanB and *vanB*, respectively (7). A *vanA* probe did not hybridize with DNA prepared from these low-level vancomycin-resistant organisms (98).

Dutka-Malen and colleagues compared the amino acid sequences of the VanA peptide and D-Ala–D-Ala ligases of *E. coli* (100). A pair of degenerate, universal PCR primers was designed against nucleotides encoding conserved groups of amino acids in these peptides. These primers could amplify DNA fragments of ca. 600 bp internal to genes encoding products related to D-Ala–D-Ala ligases (100). When *E. faecalis* V583, a prototype VanB strain (326), was used, cloning and sequencing of the resulting amplicons demonstrated that these primers amplified two distinct fragments of similar size (114). When labelled and used as a probe, one fragment hybridized with all isolates of *E. faecalis* tested, suggesting that it was part of a chromosomal gene, designated *ddl*, which encodes the usual D-Ala–D-Ala ligase (114). However, the second cloned amplicon hybridized with all enterococci with the VanB resistance phenotype but not with vancomycin-susceptible enterococci (114, 115, 309).

The DNA sequence of this proposed *vanB* gene has been determined (114, 115). The amino acid sequence of the N terminus of the predicted VanB peptide is identical to that of the 39.5-kDa vancomycin-inducible protein observed in VanB enterococci (114). There is 76% amino acid homology between the VanA ligase and the VanB peptide, further supporting the idea that *vanB* also encodes a ligase of altered substrate specificity. Gold and colleagues found that the resistance gene, designated *vanB2*, and proposed product from another VanB *E. faecalis* isolate showed 96.4% base pair and 97.5% amino acid homologies with those of *E. faecalis* V583 (140). Direct analysis of peptidoglycan precursors has confirmed the function of the VanB peptide as a ligase. The precursors of uninduced VanB cells terminate in the usual D-Ala–D-Ala dipeptide, whereas induced cells produce precursors that terminate in the depsipeptide D-Ala–D-lactate (45, 114). Thus, the biochemical basis of glycopeptide resistance in VanA and VanB enterococci would appear to be identical.

The identification of D-Ala–D-lactate in the peptidoglycan precursors in VanB enterococci (45, 114) suggests that such

strains would require *vanB* and other genes, comparable to the *van* cluster from VanA strains, in order to express resistance. Details of these other genes are not currently available. However, sequences related to *vanH* and *vanX* have been identified on either side of *vanB* (quoted in reference 96), and a vancomycin-inducible, penicillin-resistant DD-carboxypeptidase (analogous to the VanY peptide) has been detected (167). As expression of VanB resistance is inducible in most instances, it may be regulated in a similar fashion to the *van* cluster. However, some VanB strains at least do not appear to contain the *vanS* gene in PCR assays (89). Interstrain differences in regulation may explain the wide range of vancomycin MICs associated with VanB enterococci (309) and also the difference in teicoplanin susceptibility observed between VanA and many VanB strains (96).

The VanB phenotype originally described enterococci with inducible, low-level, nontransferable resistance to vancomycin but not teicoplanin (326, 409, 414). However, it has subsequently become clear that glycopeptide-resistant enterococci containing the *vanB* gene are phenotypically diverse, exhibiting a wide range of vancomycin MICs, including high-level resistance (MIC, ≤1,024 μg/ml) (309). In addition, the emergence of mutants that express *vanB* constitutively has been described both in vitro (7) and in vivo (185). These *vanB* mutants are also resistant to teicoplanin and cannot be distinguished by susceptibility testing, or other phenotypic methods, from VanA enterococci, constitutive mutants of which have also been described (157). Resistance mediated by *vanB* may also be transferable, with the gene located either on the chromosome (140, 308, 309) or on plasmids (9, 416, 417). The variable location of *vanB* suggests that it too, like *vanA*, may be associated with a transposable element. In the United Kingdom, *vanB* has recently been found on heterogeneous, transferable plasmids in enterococci isolated at four geographically diverse hospitals (416, 417). Despite this heterogeneity, a 589-bp *vanB* probe hybridized with a 2.1-kb *Eco*RV fragment on each of these plasmids (416, 417). This supports the possibility that *vanB* might be part of a larger conserved genetic element.

Low-level vancomycin resistance, but teicoplanin susceptibility, is an intrinsic property of most isolates of *Enterococcus gallinarum*, *E. casseliflavus*, and *E. flavescens* (98, 232, 281, 396). This VanC phenotype is thought to be chromosomally encoded and expressed constitutively, although recent data suggest that it may be inducible in at least some strains of *E. gallinarum* (45, 325). The use of degenerate "D-Ala–D-Ala ligase" PCR primers allowed the *vanC* gene of *E. gallinarum* BM4174 to be amplified, cloned, and sequenced, suggesting that it also encoded a ligaselike enzyme (100). A probe for *vanC* confirmed its location in highly conserved regions of the chromosome of 42 strains of *E. gallinarum* (232). The predicted VanC peptide showed 38% amino acid homology with the VanA and VanB ligases (100, 114). Peptidoglycan precursors of *E. gallinarum* terminate in the novel dipeptide D-Ala–D-serine rather than in D-Ala–D-Ala (45, 314, 325). This substitution probably reduces vancomycin binding, albeit not to the same degree as the depsipeptide found in VanA and VanB enterococci: hence, the low-level vancomycin resistance associated with *E. gallinarum* (314). Insertional inactivation of *vanC* caused reversion to vancomycin susceptibility, suggesting the existence of a second chromosomal ligase that synthesizes vancomycin-susceptible precursors (100). Indeed, the peptidoglycan precursors of this insertional mutant terminate in D-Ala–D-Ala (314).

In addition to a *vanC*-encoded D-Ala–D-Ser ligase, *E. gallinarum* also exhibits DD-dipeptidase (314) and DD-carboxypeptidase (314, 396) activities. Most of these activities are located in the cytoplasm (97 and 92%, respectively) and are not inhibited by penicillin (314). A distinct, penicillin-susceptible DD-carboxypeptidase is also present in membrane fractions and may be attributable to penicillin-binding proteins (PBPs) (314, 396). The DD-peptidase and DD-carboxypeptidase enzymes suppress synthesis of normal peptidoglycan precursors (terminating in D-Ala–D-Ala) in favor of vancomycin-resistant precursors terminating in D-Ala–D-Ser (314). When the *vanC* gene of *E. gallinarum* BM4174 was insertionally inactivated, these two other activities were no longer detected in the cytoplasm and peptidoglycan synthesis reverted to the more usual, vancomycin-susceptible pathway (314). Although the positions of the genes encoding the DD-peptidase and DD-carboxypeptidase have not been determined in relation to *vanC*, these data suggest that transcription of them is linked to transcription of *vanC*. As vancomycin resistance in strains of *E. gallinarum* may be expressed either inducibly or constitutively (325, 396), regulation will presumably vary between strains. In constitutive strains at least, expression is, by definition, not dependent on the presence of vancomycin, and so regulatory elements may differ from the *vanS-vanR* sensor-regulator system identified in inducible VanA strains.

The biochemical basis for the VanC phenotype displayed by most isolates of *E. casseliflavus* and *E. flavescens* is unknown at present. Two genes, designated *vanC-2* and *vanC-3*, which share 98.3% nucleotide identity, have been identified in these species. A probe for *vanC-2* hybridized with DNA from isolates of both *E. casseliflavus* and *E. flavescens*, confirming the relatedness of the genes (281). There is extensive similarity (66%) between the *vanC-2* or *vanC-3* gene and the *vanC* gene, now designated *vanC-1*, from *E. gallinarum*, although they do not cross-hybridize (281).

Origin of the resistance genes. There is no homology between the *vanA* or *vanB* gene and DNA from glycopeptide-susceptible enterococci (98, 114, 115, 309). This suggests that the resistance genes may have originated in another genus. Probes for *vanA* and *vanB* did not hybridize with DNA prepared from intrinsically glycopeptide-resistant lactic acid bacteria (98, 309). A *vanA* probe also failed to hybridize with DNA from *Amycolatopsis orientalis* ATCC 19795 or *Actinoplanes teichomyceticus* ATCC 31211, which produce vancomycin and teicoplanin, respectively (98). Cloning and sequencing of the teicoplanin resistance gene of *A. teichomyceticus* have confirmed that there is no nucleotide or amino acid homology with *vanA* or its product (263, 355). It therefore appears unlikely that the *van* cluster has originated in these organisms.

The base compositions (percent G+C) of DNA from *E. faecalis* and *E. faecium* are 38 and 39%, respectively (18). However, the compositions of the five genes in the *van* cluster of Tn*1546* (*vanR*, *vanS*, *vanH*, *vanA*, and *vanX*) range from 41 to 45% (18), while the *vanB* gene of *E. faecalis* V583 has an even higher G+C content of 49% (114). The deviation in the base compositions of these glycopeptide resistance genes and those of enterococcal chromosomal genes supports an extrageneric origin for the resistance genes. Interestingly, the other genes located on Tn*1546* have G+C compositions that differ from those of the *van* cluster and from each other: *vanY* (34%), *vanZ* (29%), ORF1 (37%), and ORF2 (34%) (18). This suggests that the transposon may be composed of genes from various origins (12).

Glycopeptide-dependent enterococci. Unusual variants of glycopeptide-resistant *E. faecalis* and *E. faecium* that grow only in the presence of glycopeptides have been isolated from clinical specimens in the United States (124, 158) and the United Kingdom (415). Furthermore, a glycopeptide-dependent mutant of a strain of *E. avium* has been reported (320). These are derived from enterococci of the VanA (123, 320) or VanB

(124, 158, 415) phenotype. These glycopeptide-dependent organisms are also able to grow if supplied with the dipeptide D-Ala–D-Ala (124, 158, 320), which suggests that they are unable to produce the ligase encoded by the chromosomal *ddl* gene. In the presence of vancomycin, the *vanA*- or *vanB*-encoded D-Ala–D-lactate ligase is induced, which overcomes the defect by providing an alternative means of synthesizing peptidoglycan precursors. Direct analysis of peptidoglycan precursors of vancomycin-dependent strains of *E. faecalis* and *E. avium* failed to detect normal pentapeptide-containing precursors (123, 320), lending further support to this hypothesis. Vancomycin-resistant, but nondependent, mutants arise from dependent organisms of the VanB phenotype at frequencies of 1 in 10^5 to 10^7 organisms (124, 158). These mutants are of two types: one type expresses *vanB* constitutively and is consequently resistant to teicoplanin, while the second type expresses a normal, inducible VanB phenotype (126, 158). Revertants of the latter type regain the ability to produce normal peptidoglycan precursors (123).

Staphylococci

Resistance to glycopeptides among staphylococci is phenotypically diverse, with isolates exhibiting either resistance to vancomycin or teicoplanin alone or resistance to both agents. Although to date clinical isolates of *S. aureus* have exhibited resistance only to teicoplanin (2, 3, 57, 142, 212, 218, 388), *S. aureus* strains resistant to vancomycin have been obtained in vitro either by selection of resistant mutants (86) or by the conjugal transfer of genes encoding vancomycin resistance from enterococci (288). Teicoplanin-resistant derivatives of teicoplanin-susceptible *S. aureus* strains have also been obtained in vitro (87, 212). Selection of glycopeptide-resistant CNS has also been noted in vitro (188).

Studies of glycopeptide resistance in both naturally occurring and laboratory-derived strains of *S. aureus* and CNS have provided evidence that resistance may be related to alterations in the bacterial cell wall. Transmission electron microscopy of cells of *S. aureus* (87), *S. epidermidis* (333), and *S. haemolyticus* (41) has shown that glycopeptide-resistant organisms have thicker, more irregular cell walls than do glycopeptide-susceptible organisms, although it is not clear if such modified cell walls help mediate glycopeptide resistance or are simply a phenotypic by-product of the resistance mechanism. Although some resistant isolates of *S. epidermidis* have been shown to sequester glycopeptides, particularly teicoplanin (335), other studies with clinical isolates of *S. haemolyticus* and *S. epidermidis* have shown no differences between the number of glycopeptide molecules bound by resistant and those bound by susceptible organisms (294).

Biochemical analysis of a glycopeptide-susceptible clinical isolate of *S. aureus* and a teicoplanin-resistant derivative that emerged during teicoplanin therapy in a patient with endocarditis (212) revealed the presence of a novel 35-kDa membrane-associated protein in the teicoplanin-resistant derivative (347). Further investigation showed that the teicoplanin-resistant derivative also expressed increased quantities of the PBPs PBP2-1 and PBP2-2 and was more susceptible to lysis with lysostaphin than the teicoplanin-susceptible parent organism (347). Teicoplanin-susceptible transposon insertion mutants of the teicoplanin-resistant derivative failed to produce the 35-kDa protein and produced PBP2-1 and PBP2-2 at levels comparable to those seen in the parent teicoplanin-susceptible clinical isolate (347). In each of two independent transposon insertion mutants, the Tn*551* inserts mapped to a ca. 117-kb *Sma*I fragment of the chromosome, and it was postulated that the mutation that led to emergence of teicoplanin resistance in the original clinical isolate affected the regulation of both the 35-kDa protein and the two PBP2 polypeptides (347).

A novel protein was also noted in the membrane and cytoplasmic fractions of cells of *S. aureus* which showed four- to eightfold increases in vancomycin resistance and 8- to 16-fold increases in teicoplanin resistance compared with the two clinical isolates from which they were derived in vitro (87). The glycopeptide-resistant derivatives also showed loss of reactivity with bacteriophage and anticapsular antibodies, as well as decreased susceptibility to killing by lysostaphin and lysozyme, which provided further evidence that resistance to glycopeptides was associated with changes in the bacterial cell surface (87). Although the novel protein found in the teicoplanin-resistant organisms was similar in size (39 kDa) to the inducible proteins found in glycopeptide-resistant enterococci, preliminary amino acid sequence analysis did not indicate homology with the enterococcal VanA protein (87). Interestingly, novel membrane-associated proteins of 35 and 39 kDa have also been described in teicoplanin-resistant clinical isolates of *S. haemolyticus* and *S. epidermidis*, respectively (294). The amino acid composition and degree of cross-linking of peptidoglycan of these teicoplanin-resistant organisms did not differ from those seen in glycopeptide-susceptible organisms, indicating that the 35- and 39-kDa proteins were unlikely to function as D-Ala–D-Ala ligases of altered specificity (294). Clearly, further work is needed to elucidate the mechanism(s) of glycopeptide resistance in staphylococci.

Other Gram-Positive Bacteria

Although there has been one report of a clinical isolate of *Lactobacillus viridescens* in which glycopeptide resistance was associated with the presence of plasmid DNA and production of novel cell wall proteins following exposure to subinhibitory concentrations of vancomycin (195), other studies have shown that glycopeptide resistance in lactic acid bacteria is constitutively expressed (286). It was further shown that a DNA probe for the *vanA* gene failed to hybridize with DNA from glycopeptide-resistant lactic acid bacteria (98). Such a probe also failed to hybridize with DNA from a glycopeptide-resistant isolate of *Gemella haemolysans* obtained from the blood of a pediatric patient (312).

Biochemical studies subsequently showed that resistance to vancomycin in clinical isolates of *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* was related to low levels of binding of vancomycin to native or SDS-treated cell walls (195). In strains of *Lactobacillus casei*, *Leuconostoc mesenteroides*, and *P. pentosaceus*, analysis revealed peptidoglycan precursors terminating in the depsipeptide D-Ala–D-Lac (45, 180). It was speculated that these naturally glycopeptide-resistant bacteria contained a ligase that catalyzes the formation of D-Ala–D-Lac and that they do not produce D-Ala–D-Ala (45). Analysis of the genes encoding D-Ala–D-Ala ligase-related enzymes in strains of *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, and *Lactobacillus confusus* indicated that the enzymes of these glycopeptide-resistant species were more closely related to each other (47 to 63% amino acid homology) than to the enzyme of the glycopeptide-susceptible species *Lactobacillus leichmanii* (33 to 37% homology) (107). The DNA from lactic acid bacteria did not hybridize with probes for the enterococcal *vanA* or *vanB* ligase gene (98, 309), and their ligases showed only 26 to 32% amino acid homology with the VanA and VanB enzymes (107). Therefore, although the existence of an identical resistance mechanism in enterococci and these intrinsically resistant genera suggests a possible evo-

TABLE 1. Current NCCLS zone diameter and MIC interpretive standards for glycopeptide antibiotics (279, 280)

Organisms	Antibiotic	Susceptible		Intermediate		Resistant	
		Zone diam (mm)[a]	MIC (μg/ml)	Zone diam (mm)	MIC (μg/ml)	Zone diam (mm)	MIC (μg/ml)
Enterococci	Vancomycin	≥17	≤4	15–16	8–16	≤14	≥32
	Teicoplanin	≥14	≤8	11–13	16	≤10	≥32
Other gram-positive bacteria	Vancomycin	≥12	≤4	10–11	8–16	≤9	≥32
	Teicoplanin	≥14	≤8	11–13	16	≤10	≥32

[a] All zone diameters refer to the use of 30-μg disks.

lutionary relationship (180), this is not supported by available genetic data.

DETECTION OF GLYCOPEPTIDE RESISTANCE IN THE CLINICAL LABORATORY

Criteria for Defining Phenotypic Resistance

The National Committee for Clinical Laboratory Standards (NCCLS) has recently published its latest zone diameter (280) and MIC (279) interpretive approved standards for susceptibility testing. These include recommendations for testing both vancomycin and teicoplanin (Table 1), which affirm the criteria given in an informational supplement (278) to the previous approved standards (275, 276). Different zone diameter interpretive standards are now recommended when enterococci are tested against vancomycin by disk diffusion compared with other gram-positive bacteria. For enterococci, these interpretive standards are zone diameters of ≥17 mm for susceptibility and ≤14 mm for resistance (280). These recommendations were first introduced in 1991 (277) and are based on the findings of a study by Swenson and colleagues (365) in which 100 enterococcus isolates with a range of vancomycin susceptibilities were tested by disk diffusion at four centers and the results were compared with MICs as determined by agar dilution. The NCCLS now also recommends that vancomycin inhibition zones be read with transmitted light after a full 24 h of incubation and that any haze or growth within the zone be taken into account when the zone diameter is measured (280). It is further suggested that enterococci classified as intermediate to vancomycin by disk diffusion methods be subjected to MIC determination if this agent is to be considered for treatment.

In contrast with vancomycin disk testing, the currently recommended interpretive standards for testing vancomycin by MIC methods or for testing teicoplanin by either the MIC or disk diffusion method apply equally to all gram-positive bacteria (279, 280). NCCLS interpretive standards for teicoplanin were also first given in 1991 (277) and were the same as those suggested after a study of 1,201 gram-positive clinical isolates from the United States (218), the breakpoints having been based on the correlation between MICs, zone diameters, and, in 305 cases, an assessment of the clinical outcome of teicoplanin therapy. These criteria remain unchanged in the current approved standards (279, 280) (Table 1), although there are some differences in the categories of susceptibility defined. Enterococci classified as susceptible to vancomycin or teicoplanin according to the current NCCLS zone diameter or MIC interpretive standards would have been classified as moderately susceptible by earlier recommendations (275–277). Similarly, isolates of other gram-positive bacteria for which teicoplanin MICs were 16 μg/ml had previously been classified as moderately susceptible (277) but are now classified as intermediate (278, 279).

There are differences between the current NCCLS interpretive standards and the recommendations made to define categories of susceptibility to glycopeptides in some other countries. For example, in the United Kingdom, the Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy has recommended the use of a single breakpoint concentration of 4 μg/ml to define resistance to both vancomycin and teicoplanin (422). Most U.K. laboratories use comparative disk diffusion susceptibility tests (352). No specific interpretive guidelines are given for testing glycopeptides by these methods, although 30-μg vancomycin disks are recommended. There are no recommendations made for the content of teicoplanin disks (422). The Comité de l'Antibiogramme de la Société Française de Microbiologie recommends MIC breakpoints of ≤4 and >16 μg/ml for susceptibility and resistance to both vancomycin and teicoplanin, together with an interpretive zone diameter of ≥17 mm around 30-μg disks (356). It suggests that all isolates giving zone diameters of <17 mm should be subjected to MIC determination. The Swedish Reference Group for Antibiotics recommends zone diameter interpretive standards for vancomycin of ≤14 mm (resistance) and ≥16 mm (susceptibility) for tests performed on Iso-Sensitest agar and a single MIC breakpoint concentration of 4 μg/ml (214). In contrast with recommendations made by the NCCLS, those made by the French, Swedish, and U.K. bodies make no distinction between tests performed on enterococci and those performed on other gram-positive bacteria (214, 356, 422).

Methods for Detection of Phenotypic Resistance

Despite modifications made to the criteria used to define categories of susceptibility, the actual detection of some glycopeptide-resistant organisms in the clinical laboratory remains problematic. Two studies, one in the United States (369) and the other in the United Kingdom (352), have investigated the ability of multiple clinical laboratories to detect antibiotic resistance, including resistance to vancomycin, among enterococci. These two studies have confirmed that while most laboratories can readily detect high-level vancomycin-resistant enterococci, problems may arise when enterococci with lower levels of resistance are tested. No comparable multicenter studies have been undertaken for glycopeptide-resistant staphylococci or for intrinsically resistant organisms.

Tenover and colleagues (369) reported the distribution of four glycopeptide-resistant enterococci to laboratories in New Jersey. Ninety-six percent of 76 laboratories that participated in the study correctly classified a VanA enterococcus as resistant to vancomycin (MIC, 512 μg/ml). However, fewer laboratories correctly classified as resistant two strains with the VanB or "VanB-like" phenotype. Only 17% of laboratories classified as resistant an *E. faecalis* isolate with a vancomycin MIC of 32 μg/ml, while 29% classified as resistant an *E. faecium* isolate with a slightly higher vancomycin MIC of 64 μg/ml. These

organisms were classified as moderately susceptible (in accordance with the NCCLS criteria [277] at that time) by 62 and 28% of the laboratories, respectively (369). An *E. gallinarum* strain of the VanC phenotype (vancomycin MIC, 8 μg/ml) was correctly classified as intermediate by 71% of laboratories, while the remainder of the laboratories classified it as moderately susceptible.

In a similar study undertaken in the United Kingdom, four glycopeptide-resistant enterococci were distributed to 418 laboratories as part of the U.K. National External Quality Assessment Scheme for Microbiology (352). A total of 349 laboratories returned results for vancomycin susceptibility testing. Two strains, an *E. faecalis* and an *E. faecium*, both with the VanA phenotype (vancomycin MICs, >512 μg/ml), were classified as resistant by 96 and 99% of laboratories, respectively. However, in common with the U.S. study (369), these values fell to 50 to 53% of laboratories for two *E. faecium* strains of the VanB phenotype (vancomycin MICs, 64 μg/ml). It is cause for some concern that 69 (16.5%) laboratories in the U.K. study apparently did not routinely test enterococci for resistance to vancomycin (352).

These two studies also illustrate methodological differences between vancomycin susceptibility tests performed in laboratories in the United States and those done in the United Kingdom. A disk diffusion method was used in only 4 (5.3%) laboratories in New Jersey (369), while in the United Kingdom, it was the only method used in 321 (92%) laboratories and was used in combination with other methods in 6 more laboratories (352). The majority of laboratories in New Jersey used automated or nonautomated commercial susceptibility testing systems, but these are used rarely in the United Kingdom. Many studies have shown that the methodology used can have a pronounced effect on the ability to detect glycopeptide-resistant organisms.

Disk diffusion. Glycopeptide antibiotics diffuse poorly through agar, and disks typically give small zones of inhibition. Teicoplanin has a lower diffusion coefficient (0.47 mm^2/h in Mueller-Hinton agar) than vancomycin (0.72 mm^2/h) (69). For this reason, 30-μg teicoplanin disks tend to give even smaller zones of inhibition than 30-μg vancomycin disks (69). As a consequence of poor glycopeptide diffusion, the difference between the sizes of inhibition zones for susceptible and resistant bacteria is not as great as that for some other classes of antimicrobial agents. Due to this relatively poor discriminatory power, disk diffusion tests are not a reliable means of detecting all levels of glycopeptide resistance.

Disk diffusion is effective for detecting enterococci and those intrinsically resistant gram-positive organisms that are highly resistant to vancomycin (MICs, ≥256 μg/ml). Organisms with this level of resistance usually show no zones of inhibition (diameter, 6 mm) around 30-μg vancomycin disks (341, 364, 413). Despite refinements to the NCCLS zone diameter interpretive criteria, isolates with lower levels of resistance may still be incorrectly categorized by disk diffusion. Schulz and Sahm (341) found that the current NCCLS criteria (280) allowed correct classification of 14 vancomycin-susceptible and 6 highly vancomycin-resistant (MICs, ≥256 μg/ml) enterococci. However, 10 isolates with intermediate susceptibility (MICs, 8 to 16 μg/ml) were all categorized as susceptible by disk diffusion (diameters of ≥17 mm). Furthermore, of eight isolates resistant to vancomycin (MICs, 32 to 128 μg/ml), six gave zone diameters equivalent to the interpretive standard for resistance (14 mm), while one was classified as intermediate and one was classified as susceptible (341). If the incubation time was prolonged to 48 h, a growth haze appeared inside the zone for many of these isolates, allowing categorization as resistant. In the United Kingdom, it has been suggested that the use of 5-μg vancomycin disks will allow increased detection of enterococci with low-level vancomycin resistance (413). This suggestion was supported by the U.K. National External Quality Assessment Scheme study, in which laboratories using 5-μg disks detected two VanB enterococci more readily than laboratories using disks with a higher vancomycin content (352). Use of these low-content disks has not been reported from the United States.

Similar problems have been encountered in detection of glycopeptide resistance among staphylococci by disk diffusion methods. As with enterococci, the use of 30-μg vancomycin disks can result in failure to detect resistance. Sanyal and colleagues reported an *S. epidermidis* strain resistant to vancomycin (MIC, 16 μg/ml) and teicoplanin (MIC, >8 μg/ml) but susceptible to both agents in disk diffusion tests interpreted by the Stokes comparative method (334). However, this strain was correctly classified as resistant to vancomycin when the test was repeated with a 5-μg disk (334). Several other groups have reported a poor correlation between teicoplanin zone diameters and agar dilution MICs for teicoplanin-intermediate and -resistant isolates of CNS (79, 199, 200, 218). In some instances this resulted in reports of false susceptibility (200, 218). Furthermore, susceptibility tests for teicoplanin are influenced by a variety of technical factors, including medium, inoculum size, and incubation time (79, 120).

The actual ability to detect glycopeptide-resistant staphylococci by disk diffusion is not the only problem encountered. Accurate interpretation of the results obtained can be made difficult by the use of inappropriate interpretive standards. A zone diameter of ≤14 mm on Iso-Sensitest agar was used to define resistance to vancomycin among staphylococci isolated in a regional laboratory in The Netherlands (183). This definition was based on NCCLS criteria used by Barry and colleagues (33), which referred to tests performed in accordance with NCCLS guidelines. In this study (183), 35 of 7,144 (0.5%) *S. aureus* isolates and 31 of 3,933 (0.8%) CNS gave zones sizes of ≤14 mm around 30-μg vancomycin disks and were classified as resistant. However, it is difficult to determine exactly how many of these strains were truly resistant to vancomycin. First, the tests were not performed on Mueller-Hinton agar, and the validity of applying NCCLS criteria to tests performed on Iso-Sensitest agar cannot be assessed without detailed investigation. In addition, the NCCLS interpretive standard used to define vancomycin resistance had been superseded and hence was inappropriate. While a zone size of ≤14 mm is the standard recommended to define vancomycin resistance in enterococci, a zone diameter of ≤9 mm defines vancomycin resistance in staphylococci and other nonenterococcal gram-positive bacteria (278, 280). If the data (183) are reassessed with this more rigorous NCCLS criterion, only one vancomycin-resistant isolate (an *S. haemolyticus* strain) was actually detected. Despite this, a zone diameter of ≤14 mm on Iso-Sensitest agar is the recommendation made by the Swedish Reference Group for Antibiotics (214). So, by Swedish criteria, 35 vancomycin-resistant *S. aureus* strains were indeed detected in this study. Clearly, there is still the potential for discrepant results with some zone diameter interpretive standards for staphylococci. Interpretive standards decided upon in the absence of tests of many resistant isolates often result in such problems (201, 365). As more glycopeptide-resistant staphylococci are isolated, it will be possible to reexamine and, if necessary, to modify these interpretive criteria to improve both their sensitivity and their specificity.

The E test. A variation of the traditional disk diffusion test is provided by the E test (24, 55). One advantage of this test over

disk diffusion is that it is quantitative, providing the user with a MIC for the test organism. The E test consists of a plastic strip, one side of which contains a continuous gradient of antibiotic. This strip is applied to the surface of an inoculated agar plate into which the antibiotic diffuses. During incubation, an elliptical zone of inhibition forms. The MIC is then read at the point where this ellipse touches the E-test strip (24, 55). The vancomycin MICs for vancomycin-susceptible organisms tend to be slightly higher when determined by the E test than when determined by agar dilution or broth microdilution methods (24, 330), but results still lie within the accuracy limits of these tests (± 1 $\log_2$ dilution) and do not result in the detection of false resistance (24, 199, 284, 330, 341). The E test was able to detect all levels of vancomycin resistance in enterococci and appeared to be more sensitive than disk diffusion for detecting strains for which intermediate vancomycin MICs were 8 to 16 μg/ml (341). As with disk diffusion, however, some enterococci produce a faint haze of growth within the elliptical inhibition zone, and so caution must be exercised when reading the MIC from the strip (341). It has been suggested that results for all strains not clearly susceptible to vancomycin by disk diffusion or E-test methods should be read after prolonged incubation for 48 h (341).

The use and evaluation of teicoplanin E-test strips have not been widely reported. One study found that teicoplanin MICs determined with the E test correlated well with agar dilution for a collection of clinical and reference isolates of staphylococci and enterococci (403). However, other studies have indicated that the E test gives lower teicoplanin MICs for coagulase-negative staphylococci than does agar dilution. Furthermore, this can result in reports of false susceptibility (199, 248). The use of a heavier inoculum for CNS may improve the sensitivity of both teicoplanin and vancomycin E tests (51), as may prolonged incubation.

Breakpoint screening. The use of agar plates containing a single breakpoint concentration of glycopeptides might be regarded as the ideal phenotypic method for diagnostic laboratories to screen for glycopeptide resistance. The use of a multipoint inoculator or replicator allows multiple isolates to be tested on a single plate. In addition, the method is based on agar dilution and is not subject to many of the problems associated with disk diffusion. For this reason, the results of breakpoint screening should be more reliable. However, the screening concentration should be chosen carefully in order to maximize the sensitivity and specificity of the test. Use of a concentration equivalent to the MIC interpretive standard for susceptibility (4 μg/ml [279]) may lead to a decrease in the specificity of the test at high inocula (10^6 CFU; i.e., breakthrough of susceptible colonies) (365).

A breakpoint screening method has not been used extensively in the United States, primarily because most laboratories perform susceptibility tests in accordance with NCCLS recommendations, and standardized parameters for a breakpoint test had not been published. However, Willey and colleagues found that Mueller-Hinton agar plates containing either 6 or 8 μg of vancomycin per ml provided an easy-to-read, reliable, and inexpensive means of differentiating enterococci moderately susceptible to vancomycin from those exhibiting resistance or intermediate susceptibility (408). Subsequently, another study confirmed the usefulness of this screening test for detecting all glycopeptide-resistant organisms, although sensitivity increased when brain heart infusion agar was used rather than Mueller-Hinton agar (369). Recently, this work has been reevaluated and extended. In this larger study, Swenson and colleagues also suggested that tests were easier to read if brain heart infusion agar was used (363). These workers also recommended the use of 6 μg of vancomycin per ml, an inoculum of 10^5 to 10^6 CFU, and that the test be read after a full 24 h of incubation (363). This screening technique is included in the latest NCCLS approved standard (279), although whether it will be widely adopted remains to be seen.

In the study undertaken by the U.K. National External Quality Assessment Scheme, only 13 of 349 U.K. laboratories tested enterococci for vancomycin resistance by a breakpoint method (352). Nine of these laboratories used a concentration of 4 μg/ml as recommended by the British Society for Antimicrobial Chemotherapy (422), while the other four laboratories used either 5, 8, or 10 μg/ml (352). Despite the low numbers involved, laboratories using this screening method were more likely to detect two enterococci with low-level vancomycin resistance (MICs, 64 μg/ml) than laboratories using 30-μg vancomycin disks (352).

Other methods. Traditionally, agar dilution MICs have been the standard against which to compare the ability of other methods to detect glycopeptide-resistant organisms. Broth microdilution MICs may be slightly lower, but the method correlates well with agar dilution (217, 328). Furthermore, this method allows detection of organisms such as *E. gallinarum* with low levels of vancomycin resistance (328). Broth microdilution performed in cation-adjusted Mueller-Hinton broth is a method recognized by the NCCLS (279), and it has replaced agar dilution as the reference method in some studies (218, 369, 408).

In contrast with U.K. laboratories (352), many U.S. laboratories use commercially available automated or nonautomated susceptibility testing systems. In one study, 38 of 76 (50%) laboratories in New Jersey used the Vitek system, while a 29 (38%) more laboratories used MicroScan products (369). Enterococci with the VanA phenotype are usually detected readily (328, 369), but as with disk diffusion, these systems can have difficulty in detecting some other glycopeptide-resistant organisms. This difficulty results from the short incubation times required for the tests, which may not be sufficient for the induction and expression of low levels of resistance (328, 413). Although the sensitivity of the Vitek system has been improved by the development of new software, false susceptibility is still reported for some isolates (369, 407, 408, 428), leading some workers to conclude that it is an unacceptable method for detecting vancomycin resistance in enterococci (428). MicroScan products also appear to be unable to detect all levels of vancomycin resistance (369, 408). However, visual examination of wells may increase the sensitivity of some tests performed with these products, which suggests that the problem may again be improved by alterations to the software (408). There appears to be little experience of detecting glycopeptide-resistant staphylococci with commercial systems.

Molecular Approaches

Advances in technology, particularly in the development of non-radioactively labelled gene probes and PCR, have meant that molecular techniques may now be used readily to detect antibiotic resistance genes in bacteria. In many instances, the use of DNA probes is restricted to reference laboratories, but PCR is becoming increasingly available to general diagnostic laboratories. These techniques allow laboratories to determine the genotype of an antibiotic-resistant organism in a time equivalent to, or shorter than that required to perform a traditional susceptibility test of the organism's phenotype. While these advances have a number of advantages, they also present a number of new problems.

The application of DNA probe or PCR technologies re-

quires that the genes of interest have been cloned and/or sequenced. At present, no genetic information is available concerning the genes responsible for glycopeptide resistance in staphylococci. Therefore, for the study of acquired glycopeptide resistance, these molecular techniques are currently available only for enterococci. Cloning and sequencing of the *vanA* (54), *vanB* (114), *vanC-1* (100), and *vanC-2* (281) genes have enabled probes for each to be made. Furthermore, several workers have used the published gene sequences to develop PCR assays that produce a variety of gene-specific amplicons (for example, see references 81, 97, 406, 416, and 418). In some laboratories, the amplicons produced have been used directly to assign a genotype to glycopeptide-resistant enterococci (81, 97), while in others the amplicons have been labelled and subsequently used as gene probes (406, 416, 418).

By defining the genotype of glycopeptide-resistant enterococci, molecular techniques may indicate strains in which gene expression is unusual (81, 157, 185, 309), or which contain multiple resistance genes (95, 363), or even strains with potentially novel resistance genes (81). Phenotypic tests of susceptibility do not permit recognition of these anomalous strains. For example, some enterococci of the *vanB* genotype are resistant to teicoplanin as well as to vancomycin. These organisms may be mutants that express *vanB* constitutively (7, 185), or they may retain inducible expression (89). They cannot be distinguished in susceptibility tests from enterococci of the VanA class. Molecular techniques can detect the *vanB* gene in such organisms and thus distinguish them from true *vanA* enterococci (81, 89, 185).

Clark and colleagues reported a strain of *E. raffinosus* that exhibited intermediate vancomycin resistance (MIC, 16 μg/ml) but was susceptible to teicoplanin (MIC, 1 μg/ml). The strain yielded a 1-kb *vanA*-specific product in a PCR assay and carried a 34-kb plasmid which hybridized with a probe for the *vanA* gene (81, 82). This plasmid was similar in size to those carrying the *vanA* gene in vancomycin-resistant isolates of *E. faecalis* and *E. faecium* isolated at the same hospital. However, isolates of these species had typical VanA phenotypes; i.e., they were highly resistant to vancomycin and teicoplanin, suggesting altered regulation of the *vanA* gene in the *E. raffinosus* strain (81). In other studies, the use of PCR and DNA probes indicated that atypical isolates of *E. gallinarum* and *E. casseliflavus* that were highly resistant to both vancomycin and teicoplanin contained the *vanA* gene cluster in addition to the *vanC-1* or *vanC-2* gene characteristic of these species (95, 363). Enterococci which have the VanA phenotype but which do not yield PCR products with *vanA*, *vanB*, or *vanC-1* primers have also been isolated (81, 363). This might indicate significant sequence divergence from these resistance genes in the regions of the PCR primers, or alternatively, the isolates may carry previously unrecognized glycopeptide resistance genes.

When the *vanA* gene was first cloned (54), a probe was prepared and was specific for organisms of the VanA phenotype (98). At this time, the VanB phenotype was usually associated with low-level resistance to vancomycin (typical MICs, ≤64 μg/ml) (326, 409, 414). However, it is now recognized that some VanB enterococci are resistant to much higher levels of vancomycin (MICs, ≤1,024 μg/ml) (309), levels that were originally associated only with the VanA phenotype. Several recent studies suggest that *vanA* DNA probes may, in some instances, no longer be a reliable means of distinguishing all *vanA* and *vanB* organisms (81, 406, 417, 420). Willey and colleagues used a 1-kb PCR product as a probe for the *vanA* gene (406). Although this probe hybridized with enterococci of the VanA phenotype, it also hybridized strongly with strains for which vancomycin MICs were 512 to 1,024 μg/ml and teicoplanin MICs were 8 μg/ml. The latter strains are classified as susceptible to teicoplanin by NCCLS criteria (279), giving rise to an apparent anomaly between their phenotype (VanB) and genotype. However, they would be regarded as resistant (or intermediate) to teicoplanin in the United Kingdom (422), France (356), and Sweden (214) and assigned the VanA phenotype, which would be consistent with the hybridization results. This 1-kb *vanA* probe also hybridized, albeit faintly, with VanB isolates of *E. faecium* highly resistant to vancomycin (MICs, 256 to 512 μg/ml) and fully susceptible to teicoplanin (MICs, ≤0.5 μg/ml) (406). These isolates remain anomalous by all criteria. Clark and colleagues also noted two VanB isolates of *E. faecium* for which the MICs of vancomycin and teicoplanin were 32 to 64 and 0.25 to 1 μg/ml, respectively, and which hybridized with a 698-bp *vanA* gene probe (81). Similarly, *E. faecium* strains which had the VanB phenotype, but which hybridized with a 399-bp *vanA* probe, have also been isolated in the United Kingdom (417, 420). Despite hybridization with *vanA* probes, these U.K. isolates and those reported in the United States by Clark and colleagues were shown in PCR assays to contain the *vanB* gene and not *vanA* (81, 417). It is of interest that these workers have observed nonspecific hybridization between their *vanA* probes and some enterococci that have the VanB phenotype and a confirmed *vanB* genotype. The 698- and 399-bp *vanA* probes used showed good specificity, failing to hybridize with most VanB enterococci (81, 418). It is possible, therefore, that the nonspecific hybridizations observed reflect greater sequence similarity between the *vanB* genes of a few VanB enterococci and the *vanA* gene (81).

In some instances, the detection of glycopeptide resistance genes may also clarify the identification of the enterococcus. Identification of *E. gallinarum* may be achieved with a probe (232) or PCR assay (97) specific for the *vanC-1* gene, which is found only in this species. *Enterococcus* strain BM4172, which exhibits low-level vancomycin resistance, was originally identified as *E. faecium* (116) but was subsequently shown to carry *vanC-1*. Further physiological tests undertaken to explore this apparent anomaly confirmed the identity of the strain as *E. gallinarum* (232). Similarly, a probe (281) or PCR assay (97) for the *vanC-2* or *-3* gene may be used for the identification of *E. casseliflavus* and *E. flavescens.*

It is unlikely that molecular techniques will ever replace phenotypic susceptibility testing. Indeed, this may even be regarded as undesirable. It is preferable for these approaches to be used in parallel. A combination of a phenotypic and a genotypic test can identify anomalies for individual strains, such as nonexpression of resistance genes, and can confirm the identification of some species. At present, PCR assays are available only for detection of the glycopeptide resistance genes found in enterococci. However, when sequence data are forthcoming, similar assays will be possible for glycopeptide resistance genes in other genera, including staphylococci.

GEOGRAPHICAL DISTRIBUTION OF GLYCOPEPTIDE-RESISTANT BACTERIA

Enterococci

Glycopeptide-resistant enterococci have been reported from many parts of the world, including Belgium, France, Germany, Italy, The Netherlands, Spain, the United Kingdom, and the United States. The reported global distribution of glycopeptide-resistant enterococci at the time of writing is summarized in Tables 2 and 3. In the United Kingdom, where the first nosocomial outbreak of glycopeptide-resistant enterococci occurred in the late 1980s (383, 384), there is evidence that the

TABLE 2. European countries in which glycopeptide-resistant enterococci have been isolated

Country	Organism	Phenotype	Reference(s)
Belgium	*E. casseliflavus*	VanA	95
Belgium	*E. faecalis*	VanA	95
Belgium	*E. gallinarum*	VanA	95
France	*E. faecium*	NR[a], VanB	48, 303
Germany	*E. faecium*	VanA	221
Italy	*E. faecalis*	VanA	247, 389
The Netherlands	*E. faecalis*	VanA/B[b]	164, 299
The Netherlands	*E. faecium*	VanA/B	164
Spain	*E. durans*	VanA	374
Spain	*E. faecalis*	VanA/B	70, 297
Spain	*E. faecium*	VanA/B	70, 239, 374
United Kingdom	*E. avium*	VanA	311
United Kingdom	*E. durans*	VanA	170
United Kingdom	*E. faecalis*	VanA	130, 419
United Kingdom	*E. faecium*	VanA/B	130, 207, 242, 398, 411, 419
United Kingdom	*E. flavescens*	VanC	419
United Kingdom	*E. gallinarum*	VanC	419

[a] NR, not reported for some isolates.
[b] VanA/B, both VanA and VanB phenotypes reported.

number of hospitals affected by such resistant organisms has increased in recent years. Between 1988 and 1993, the Antibiotic Reference Unit of the Public Health Laboratory Service received over 300 isolates of glycopeptide-resistant enterococci from 30 different U.K. hospitals, with the number of hospitals referring isolates increasing from 1 in 1988 to 18 in 1993 (267). Of the 30 affected hospitals, 5 experienced large outbreaks (>30 isolates), which sometimes persisted for more than 6 months (267).

There is also evidence that the prevalence of enterococci resistant to glycopeptides has increased in the United States in recent years. A study of 705 enterococci isolated between July 1988 and April 1989 from eight tertiary-care hospitals in six regions of the United States revealed only two isolates (0.3%) that were resistant to vancomycin (147). Similarly, data from the Centers for Disease Control and Prevention National Nosocomial Infections Surveillance system showed that only 0.3% of nosocomial enterococci were resistant to vancomycin in 1989 (10). However, data from this system showed that by 1993 the proportion of nosocomial enterococci resistant to vancomycin had increased over 20-fold, to 7.9% (10). An increase in glycopeptide-resistant enterococci was particularly noted in intensive care units (ICUs), in which the proportion of resistant isolates increased from 0.4% in 1989 to 13.6% in 1993 (10). A similar trend was noted by the New York City Department of Health, which reported that while glycopeptide-resistant enterococci were isolated from only 2 patients in New York City in 1989, such organisms were obtained from approximately 250 patients during the first 9 months of 1991 (132).

Glycopeptide-resistant enterococci were found in hospitals in 9 of the 33 states participating in the National Nosocomial Infections Surveillance program, with the highest percentage of resistant isolates occurring in hospitals in New York (8.9%), Pennsylvania (5.6%), and Maryland (3.6%) (10). In a separate report from the Centers for Disease Control and Prevention published in 1993, it was further noted that glycopeptide-resistant enterococci were isolated in 31 hospitals in 14 states (81). The states in which glycopeptide-resistant enterococci have been reported to date are listed in Table 3.

Staphylococci

To date, there have been no reports of resistance to vancomycin among clinical isolates of *S. aureus*, although isolates with low-level resistance to teicoplanin have been reported from France (57, 142, 388) and the United States (2, 212, 218). Resistance to glycopeptides, particularly teicoplanin, is relatively more common among CNS, with such isolates having been reported from France, Germany, Spain, the United Kingdom, and the United States. Among the CNS, glycopeptide resistance has been noted in the species *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. warneri*, and *S. xylosus* (Table 4).

Lactic Acid Bacteria

Resistance to glycopeptides has been noted to occur commonly among lactic acid bacteria belonging to the genera *Lactobacillus*, *Leuconostoc*, and *Pediococcus* (203). Organisms belonging to these genera are commonly associated with products such as dairy produce, vegetables, fruit, plants, and alcoholic beverages, but they have also been isolated from humans. Although isolates from humans may reflect commensal organisms that have colonized the digestive or genitourinary tract

TABLE 3. States in the United States in which glycopeptide-resistant enterococci have been isolated

State	Organism	Phenotype	Reference(s)
Alabama	*E. faecium*	VanB	81
California	*E. faecium*	NR[a], VanA	81, 128
California	*E. gallinarum*	VanC	396
Connecticut	*E. faecium*	VanA	81, 324
District of Columbia	*E. faecium*	VanB	81
Delaware	*Enterococcus* sp.	NR	379
Georgia	*E. faecium*	VanA	81
Georgia	*E. gallinarum*	VanC	81
Illinois	*E. faecium*	VanA/B[b]	80, 81, 185, 290, 324
Illinois	*E. gallinarum*	VanC	396
Louisiana	*E. faecium*	VanB	81
Maryland	*Enterococcus* sp.	VanB	81
Maryland	*E. faecalis*	VanB	81
Maryland	*E. faecium*	VanB	81, 149
Michigan	*E. faecium*	VanB	80
Michigan	*E. gallinarum*	VanC	81
Minnesota	*E. faecium*	VanB	81
Missouri	*E. faecalis*	VanB	81
New Hampshire	*E. faecium*	VanA	324
New Jersey	*E. faecalis*	VanA	81
New Jersey	*E. faecium*	VanA	81, 292, 324
New Jersey	*E. gallinarum*	VanC	81
New Jersey	*E. raffinosus*	VanB[c]	81, 82
New York	*E. faecalis*	VanA/B	53, 178, 408
New York	*E. faecium*	VanA/B	53, 81, 178, 181, 215, 261, 322, 324, 408
New York	*Enterococcus* sp.	VanA/B	132
Ohio	*Enterococcus* sp.	NR	402
Pennsylvania	*E. casseliflavus*	VanC	156
Pennsylvania	*E. faecalis*	VanB, NR	156, 379
Pennsylvania	*E. faecium*	VanB, NR	80, 156, 236, 379
Pennsylvania	*E. gallinarum*	VanC	156, 396
Rhode Island	*E. faecium*	VanB	52
South Carolina	*E. casseliflavus*	VanC	81
South Carolina	*E. gallinarum*	VanC	81
Virginia	*E. faecium*	VanA	324

[a] NR, not reported for some isolates.
[b] VanA/B, both VanA and VanB phenotypes reported.
[c] Isolate shown to carry the *vanA* gene.

TABLE 4. Countries from which glycopeptide-resistant staphylococci have been reported

Country	Organism	Phenotype[a]	Reference(s)
France	*S. aureus*	T	57, 388
France	*S. epidermidis*	T	142
France	*S. haemolyticus*	T	57, 142
France	CNS	T	142, 251, 388
Italy	*S. haemolyticus*	T	41
Spain	*S. epidermidis*	T	72
Spain	*S. haemolyticus*	T	72
Spain	*S. hominis*	T	72
United Kingdom	*S. epidermidis*	T/TV	294, 334
United States	*S. aureus*	T	2, 212, 218
United States	*S. epidermidis*	T/T(V)	2, 26, 218
United States	*S. haemolyticus*	T/V	2, 26, 218, 387
United States	*S. hominis*	T	2
United States	*S. warneri*	T	2, 26
United States	*S. xylosus*	T	2
United States	CNS	T	205

[a] T, teicoplanin resistance; V, vancomycin resistance.

(156, 203), there are a number of reports of the isolation of such organisms from clinically significant sites, including blood (20, 40, 86, 143, 144, 162, 176, 249, 250, 317, 351, 364), cerebrospinal fluid (133, 364), urine (249, 364), peritoneal fluid (317, 332, 364), wounds, and abscesses (27, 249, 317, 364). Lactobacilli have been reported from cases of endocarditis involving either native (162) or prosthetic (20) heart valves, although in the latter report resistance to glycopeptides was not commented on. Other settings in which lactobacilli have been reported to have a pathogenic role include cases of pneumonia (358) and a patient with a thumb abscess (27). In many cases of leuconostoc and pediococcal bacteremia, patients were probably predisposed to infection by virtue of underlying illness or immunosuppression (176, 317). The geographical distribution of glycopeptide-resistant lactic acid bacteria isolated from humans is shown in Table 5.

Other Gram-Positive Bacteria

Resistance to glycopeptides has been documented in clinical isolates of *Erysipelothrix rhusiopathiae*, an organism that may infect humans following exposure to animals or contaminated animal products. In the 1980s, Gorby and Peacock reported resistance to the inhibitory and bactericidal activity of vancomycin in five isolates of *E. rhusiopathiae* (145). Similar results were reported subsequently by other investigators, with vancomycin and teicoplanin MICs and MBCs commonly falling in the range of 8 to >64 and 32 to >64 μg/ml, respectively (342, 390, 391). Countries where vancomycin-resistant *E. rhusiopathiae* have been documented include the United States (342), the United Kingdom (186), and Italy (390, 391).

There is also increasing evidence that resistance to glycopeptides may be an inherent trait of *Nocardia* spp., as 5 clinical isolates of *Nocardia* spp. obtained in Denmark (202) and 12 clinical isolates of *Nocardia asteroides* isolated in France (168) all appeared resistant to both vancomycin (MICs, 8 to >256 μg/ml) and teicoplanin (MICs, 32 to >256 μg/ml). In another study of isolates from France, 35% of 40 isolates of *N. asteroides* and 60% of 15 isolates of *N. farcinica* were resistant to vancomycin (50). However, resistance to vancomycin was assessed with 30-μg disks, which raises the possibility that some of the isolates classified as vancomycin susceptible might actually have exhibited low-level resistance, which would have been difficult to detect with this screening method.

There has been one report from the United Kingdom of the isolation from the blood of a pediatric patient of *G. haemolysans* resistant to both vancomycin (MIC, >32 μg/ml) and teicoplanin (MIC, >32 μg/ml) (312). This isolate was unusual in that most *Gemella* isolates have antimicrobial susceptibility patterns similar to those of viridans streptococci and are susceptible to vancomycin (312). Torres and colleagues also reported high-level glycopeptide resistance in an isolate of *Gemella morbillorum* (374). There have also been reports of glycopeptide resistance in bacteria identified as *Arcanobacterium haemolyticum* (130), *Corynebacterium* spp. (28, 130, 134), *Lactococcus* spp. (130, 374), *Oerskovia* spp. (130), and *Streptococcus milleri* (374).

EPIDEMIOLOGY OF NOSOCOMIAL GLYCOPEPTIDE-RESISTANT BACTERIA

Enterococci

Until recently, the epidemiology of enterococcal infections was poorly understood due to a lack of discriminatory typing schemes. However, the application of molecular methods to clusters of glycopeptide-resistant enterococci has provided a greater insight into this problem. These methods have included the analysis of restriction fragment length polymorphisms of total DNA (48, 132, 170, 207, 378), ribotyping (48, 207, 265, 268, 322, 418, 420), various pulsed-field gel electrophoresis techniques (52, 53, 80, 81, 268, 269, 324), analysis of intact (81, 247) or digested (52, 80, 236, 269) plasmid DNA, and digestion

TABLE 5. Isolation of lactic acid bacteria from humans

Country	Organism	Reference(s)
Argentina	*Lactobacillus confusus*	27
Australia	*Pediococcus acidilactici*	144
Belgium	*Lactobacillus* spp.	244
Belgium	*Leuconostoc* spp.	244
Belgium	*Pediococcus acidilactici*	244, 366
Belgium	*Pediococcus pentosaceus*	244, 366
France	*Lactobacillus rhamnosus*	78
France	*Leuconostoc lactis*	29
France	*Leuconostoc mesenteroides*	29
France	*Leuconostoc paramesenteroides*	29
France	*Pediococcus acidilactici*	29, 250, 351, 366
France	*Pediococcus pentosaceus*	29, 366
Ireland	*Pediococcus pentosaceus*	86
Nigeria	*Leuconostoc mesenteroides*	196
South Africa	*Leuconostoc mesenteroides*	133
Spain	*Lactobacillus* sp.	382
Spain	*Leuconostoc citreuni*	40
United Kingdom	*Lactobacillus acidophilus*	358
United Kingdom	*Lactobacillus fermentum*	358
United Kingdom	*Lactobacillus jensenii*	20
United Kingdom	*Lactobacillus rhamnosus*	332
United Kingdom	*Leuconostoc mesenteroides*	143
United Kingdom	*Leuconostoc paramesenteroides*	143
United States	*Lactobacillus acidophilus*	162
United States	*Lactobacillus casei*	162
United States	*Lactobacillus confusus*	156, 317, 364
United States	*Lactobacillus* sp.	156, 364
United States	*Leuconostoc citreum*	364
United States	*Leuconostoc lactis*	364
United States	*Leuconostoc mesenteroides*	176, 317, 364
United States	*Leuconostoc paramesenteroides*	176, 364
United States	*Leuconostoc* sp.	364
United States	*Pediococcus acidilactici*	249, 317, 364
United States	*Pediococcus pentosaceus*	156, 364

of amplified fragments of glycopeptide resistance genes (124, 378, 393).

Comparative studies have suggested that ribotyping is less discriminatory than restriction fragment length polymorphisms of total DNA, although the use of more than one restriction enzyme may increase the level of discrimination obtained with the former method (48, 171). It has also been reported that while ribotyping may be useful for species discrimination (171), pulsed-field gel electrophoresis is preferable for the discrimination of strains (146). Several workers have suggested that combinations of molecular methods, such as pulsed-field gel electrophoresis and analysis of plasmid DNA (93) or analysis of chromosomal restriction fragment length polymorphisms, plasmid DNA, and use of a resistance gene probe (269, 418), may lead to a better understanding of the epidemiology.

For these molecular typing methods, there is no consensus regarding the number of band differences required to discriminate between strains. For instance, Chow and colleagues (80) considered isolates to be similar if their pulsed-field gel electrophoresis patterns differed by no more than one band, while others have taken differences of two (81, 324) or five (273) bands to indicate clonal relatedness. It is to be hoped that further work will allow standardization of the interpretive criteria applied to these molecular methods.

Clusters of glycopeptide-resistant enterococci. Although there have been a number of sporadic cases of infection caused by glycopeptide-resistant enterococci (170, 297, 311), most reports have described clusters of cases involving either a single or multiple strains of *E. faecalis* or, in particular, *E. faecium*. The occurrence of such clusters has highlighted not only that certain strains are able to spread widely between patients but also the potential of enterococci to disseminate glycopeptide resistance genes.

The contrast between sporadic and epidemic glycopeptide-resistant enterococci was noted in a 42-month study of around 5,000 fecal samples from hospitalized cancer patients. During the first 35 months, only sporadic enterococci of the VanA, VanB, and VanC phenotypes were identified, but subsequently a single strain was isolated from 23 patients (269). The spread of single strains of glycopeptide-resistant enterococci has been reported from a number of other centers (52, 181, 207, 236, 247, 265, 266, 268, 322, 372, 418, 420). The majority of these clusters have been confined to single hospital units, although some have spread to a number of units. For example, two hospitals have reported single strains of multiresistant *E. faecium* that spread to 8 and 10 wards (52, 163). Similarly, in a Spanish hospital, a strain of glycopeptide-resistant *E. faecalis* was recovered over a 4-year period from patients on 10 different units (265). In one noteworthy instance, *E. casseliflavus*, which is a rare human pathogen, was responsible for an outbreak of bacteremias on a hemodialysis unit (238).

Interhospital spread of glycopeptide-resistant strains of *E. faecium* has also been reported (80, 81, 324) and was, in some instances, attributable to transfer of patients (91). However, caution should be exercised in interpreting these results since the isolation of identical types from epidemiologically unrelated sources may indicate the existence of widespread clones. To resolve this question, molecular methods will need to be applied more widely to allow in-depth analysis of enterococcal population genetics.

In many clusters, multiple strains of glycopeptide-resistant enterococci are identified. In one study, glycopeptide-resistant *E. faecium* strains were isolated from 15 patients on four wards over a 17-month period (48). Although the hospital stay of many of the patients overlapped, the isolates were unrelated, suggesting that interstrain dissemination of resistance genes had occurred. More direct evidence has come from the use of *vanA* gene probes to demonstrate spread of resistance plasmids between distinct strains (70, 269, 418). Frieden et al. (132) concluded that the rapid spread of glycopeptide-resistant enterococci in 361 patients at 38 New York hospitals over a 2-year period was due to the dissemination of a highly mobile genetic element. The ability of the genetic element to mobilize was demonstrated when the same genetic material was found at different locations on the chromosome of identical strains from the same hospital. In vitro transfer of glycopeptide resistance genes both within and between species of enterococci has been well documented (231, 287, 384).

Origin of glycopeptide-resistant enterococci. It has been suggested that glycopeptide-resistant enterococci originate in hospitals and then spread to the community (132). However, a glycopeptide-resistant *E. avium* strain was isolated from a patient at a district general hospital in which vancomycin had not been used in the preceding 6 months (311). Furthermore, a glycopeptide-resistant *E. faecium* strain was isolated from an outpatient with no recent history of hospitalization or exposure to antibiotics (149). These findings suggest that the antibiotic pressure that occurs in hospitals is not necessary for the emergence of glycopeptide resistance in enterococci.

Such anomalies are compatible with the suggestion that glycopeptide-resistant enterococci are ubiquitous in community, sewage, and animal sources. Enterococci are part of the normal bowel flora, and several studies have reported the isolation of glycopeptide-resistant enterococci from sewage (35, 221, 374). Of potentially greater significance is the isolation of *E. faecium* (*vanA* genotype) from farm animals (including a duck, a chicken, a turkey, two pigs, a pony, and a dog) and from each of five uncooked chickens bought from retail outlets (35). Some human isolates were indistinguishable from isolates derived from nonhuman sources, which might suggest that glycopeptide-resistant enterococci from animals may pass to humans via the food chain (35). A recent study reported the isolation of *E. faecium* with the *vanA* genotype from poultry and pig farms in Germany on which avoparcin, a glycopeptide antibiotic not used in humans, was used as a growth-promoting agent (220). In contrast, no glycopeptide-resistant enterococci were isolated from farms not using this agent. Avoparcin is approved for use as a feed additive in the United Kingdom but not in the United States (427). In some countries, the agricultural use of avoparcin may therefore provide a selective pressure for the emergence of glycopeptide resistance in enterococci colonizing farm animals. These animals may be an important reservoir of glycopeptide-resistant enterococci for humans. Markers that distinguish enterococci isolated from human and animal sources would allow this possibility to be investigated. One such marker in *E. faecium* might be the production of acid from raffinose, as it has been suggested that isolates positive in this test are of poultry origin (92). Raffinose fermentation might therefore be explored further as a possible marker for the spread of *E. faecium* from poultry to humans. Interestingly, raffinose-positive, glycopeptide-resistant *E. faecium* strains have been observed in at least two nosocomial outbreaks in the United Kingdom (264).

Colonization of patients and staff. The presence of glycopeptide-resistant enterococci in a hospital is usually detected when these isolates are involved in clinically significant infections. The extent of carriage and contamination with glycopeptide-resistant enterococci becomes apparent only when surveillance cultures of patients, staff, and the environment are undertaken. Rates of fecal colonization by glycopeptide-resistant enterococci reflect the location of the patient (207). Screening of 354 fecal samples revealed glycopeptide-resistant

E. faecium in 15% of renal patients, 5% of patients on other hospital units, and 2% of community (general practice) patients (207). On ICUs and hematology and renal units containing patients infected with glycopeptide-resistant enterococci, colonization rates of 10 to 33% have been reported (128, 164, 181, 207, 215, 236), and similar rates were noted among nursing home residents, not all of whom had been recently hospitalized (128). Nursing homes might therefore act as a reservoir of glycopeptide-resistant enterococci that might subsequently spread to hospitals. Fecal colonization rates of 75% have been reported for *E. gallinarum* and *E. casseliflavus* among pediatric liver transplant patients (156).

Differences in rates of fecal carriage between studies might be due in part to methodological differences. In three separate studies, fecal or rectal swabs were reported to be the best means of detecting glycopeptide-resistant enterococci (181, 236, 322). In one of these studies, all of the patients with positive rectal cultures also had a positive groin culture (181). In contrast, glycopeptide-resistant *E. faecalis* was not detected in the feces of eight infected patients on a neurosurgical ICU, although in five patients colonization of the oropharynx or trachea was detected (247). In one study comparing five different sites (rectum, perineum, mouth, throat, and nose) for detection of glycopeptide-resistant enterococci, rectal swabs alone had a sensitivity of 61%, but this increased to 76% when combined with perineal and mouth swabs (397). The use of enrichment culture in antibiotic-containing broth prior to plating may also increase the sensitivity of screening (319, 322). In another study, the inclusion of clindamycin in a selective medium to inhibit the growth of lactobacilli permitted rapid detection of glycopeptide-resistant enterococci in stool specimens (102).

Repeated fecal sampling has shown that patients often remain colonized for several months (164, 236, 322). In one instance, a patient readmitted to hospital after 12 months still carried the epidemic strain in the feces (52), suggesting that some strains of glycopeptide-resistant enterococci can become established as part of the normal bowel flora in the absence of selective antimicrobial pressure. A correlation between the presence of $\geq 10^6$ CFU/ml in a single fecal sample and an increased risk of persistent bowel colonization has been reported (156). Such persistence leads to the potential for transfer of glycopeptide resistance genes in vivo to other commensal bowel flora (130) or to other bacteria such as MRSA, CNS, and JK corynebacteria to which vancomycin frequently has relevance for treatment (see below). Noble and colleagues (288) demonstrated that *vanA*-mediated glycopeptide resistance can be transferred in vitro from *E. faecalis* to a methicillin-susceptible strain of *S. aureus.*

For these reasons, there is a need to find methods of clearing glycopeptide-resistant organisms from the feces of colonized individuals (130, 292). The efficacy of oral regimens of vancomycin or bacitracin has been evaluated (292). Glycopeptide-resistant enterococci were no longer detected in the feces of 42% (8 of 19) of patients receiving oral vancomycin compared with all eight patients receiving oral bacitracin, although the organism recurred after therapy in two of the patients receiving bacitracin. Novobiocin has also been suggested for eradication of gastrointestinal colonization of glycopeptide-resistant enterococci because of the high levels achievable in feces (394). However, others have suggested that high carriage rates preclude realistic attempts to eradicate glycopeptide-resistant enterococci from the feces of colonized patients (207).

A strain of glycopeptide-resistant *E. faecium* was able to survive for 30 min on experimentally inoculated hands (399), which suggests that ward personnel may be involved in the transmission of glycopeptide-resistant enterococci between patients. Indeed, staff carriage has been implicated previously in the transmission of a β-lactamase-producing strain of *E. faecalis* (315). Although a number of studies have investigated colonization of staff by glycopeptide-resistant enterococci during outbreaks, the findings have been inconsistent. Two studies have failed to detect glycopeptide-resistant enterococci on the hands of staff caring for infected patients (322, 384). In one of these studies, 1 of 41 staff carried glycopeptide-resistant enterococci in feces, although this was negative on repeat culture (322). In contrast, others have demonstrated fecal colonization with the outbreak strain of 7% of staff on a medical-surgical ICU (181).

Environmental contamination. Glycopeptide-resistant isolates of both *E. faecium* and *E. faecalis* survived on a variety of hospital environmental surfaces, including telephones and stethoscopes, for at least 1 h and could be recovered from bed rails and countertops for up to 3 and 7 days, respectively (359). These observations suggest that the environment is a potential reservoir for nosocomial infections with glycopeptide-resistant enterococci. A number of investigators have sampled environmental surfaces in units where infected or colonized patients were located. In some studies no environmental contamination was detected (181, 322, 418), while in others glycopeptide-resistant enterococci were isolated from a variety of surfaces, including monitoring dials and doorknobs (both of which might be consistent with hand carriage) (52, 215). Interestingly, glycopeptide-resistant enterococci were isolated more frequently from the environment of patients with diarrhea (46%) than from that of those without diarrhea (15%). In patients without diarrhea, positive samples were found only on surfaces in close proximity (patient gowns, bed linen, and bed side rails), while when patients had diarrhea, a greater variety of surfaces were contaminated, including intravenous pump, electrocardiogram monitor, over bed tables, floor, blood pressure cuffs, pulse-oximeter coupling, stethoscope, and a bathroom door (52). Thus, unreported variations in the numbers of patients with diarrhea may explain why glycopeptide-resistant enterococci were recovered from environmental surfaces in some studies but not in others.

In an outbreak of glycopeptide-resistant *E. faecium* on a medical-surgical ICU, the epidemic strain was isolated repeatedly from the rectal probe handles of three electronic thermometers used exclusively on the unit (236). Disposable probe sheaths intended to protect the instrument from contamination were not satisfactory, as soiling of the probe handle occurred. Similarly, inadequate disinfection of bedpans has been associated with cross-infection by glycopeptide-resistant enterococci (73, 129). In another hospital a rented, low-airloss therapeutic bed was implicated as the route by which glycopeptide-resistant enterococci were introduced (296). These studies highlight the need for cleaning and decontamination procedures that should be sufficient to destroy relatively heat-stable enterococci. However, further studies are required to establish conclusively whether environmental contamination of surfaces is directly involved in transmission of glycopeptide-resistant enterococci between patients or is simply the result of shedding of bacteria by colonized or infected patients.

Risk factors for acquisition of glycopeptide-resistant enterococci. The three risk factors for acquisition of glycopeptide-resistant enterococci identified most often are prior vancomycin therapy, length of stay in hospital, and administration of broad-spectrum antibiotics, especially cephalosporins. Use of multiple antibiotics in patients colonized with low numbers of glycopeptide-resistant enterococci would allow enterococcal overgrowth with increased risk of infection and dissemination

(261). Other risk factors that have been reported are intermittent dosing of vancomycin in patients with renal insufficiency (181), prior infection (215), duration of ceftazidime therapy (236), proximity to affected patients and care by colonized staff (52), the use of invasive procedures (53), and selective decontamination of the digestive tract (247).

Staphylococci

Staphylococci with low-level glycopeptide resistance have, in many cases, been isolated from individual patients who have received glycopeptide therapy, sometimes for prolonged periods. In one instance, a neutropenic patient treated with vancomycin for several weeks and who was colonized in the throat and bowel with glycopeptide-susceptible *S. haemolyticus*, subsequently developed bacteremia with *S. haemolyticus* showing intermediate resistance to vancomycin (MIC, 8 to 16 μg/ml) (387). Organisms with intermediate resistance to vancomycin were also isolated from a tracheal aspirate. Similarly, *S. haemolyticus* resistant to both vancomycin (MIC, 8 μg/ml) and teicoplanin (MIC, 64 μg/ml) was isolated from knee fluid from a patient treated with teicoplanin for 43 days (21). In another case, serial isolates of *S. aureus* from the blood of a patient unsuccessfully treated with teicoplanin for endocarditis showed an eightfold increase in resistance to teicoplanin (212). Treatment failure with teicoplanin was also documented in two neutropenic patients infected with teicoplanin-resistant *S. aureus* (MIC, 8 μg/ml) and *S. haemolyticus* (MIC, 16 μg/ml), respectively (57). Interestingly, both isolates remained susceptible to vancomycin, and the patients responded to therapy with this agent (57). Peritonitis due to *S. epidermidis* resistant to both vancomycin and teicoplanin has been described in a continuous ambulatory peritoneal dialysis (CAPD) patient who had received multiple courses of vancomycin (336).

In addition to colonization or infection of individual patients, cross-infection with glycopeptide-resistant staphylococci has been noted. During a 12-month period, MRSA organisms with reduced susceptibility to teicoplanin (MICs, 8 to 16 μg/ml) were isolated from 12 patients in a hospital in France (245). Characterization of the isolates by field inversion gel electrophoresis revealed the presence of three groups, with one group comprising seven epidemiologically-related isolates (245). This observation highlights the need for surveillance of nosocomial isolates of staphylococci for resistance to glycopeptides.

ANTIBIOTIC TREATMENT OF INFECTIONS CAUSED BY GLYCOPEPTIDE-RESISTANT GRAM-POSITIVE BACTERIA

Glycopeptide resistance becomes of clinical significance when known or suspected hypersensitivity in the patient, or resistance to favored agents in the pathogen, drives treatment towards glycopeptides. This arises either in response to a defined infection or in empirical use (for example, in peritonitis associated with CAPD or infection of intravascular devices) or prophylaxis in similar situations. Fortunately, glycopeptide resistance has not yet been reported in MRSA or highly β-lactam-resistant pneumococci, in which it would present obvious and serious difficulties in management. It is clear, however, that challenging situations are occurring with greater frequency, particularly with infections by *Enterococcus* spp., to some extent with CNS, and occasionally with other species. The prominence given in this review of treatment to the various species will reflect the perceived clinical importance of glycopeptide resistance in them. In particular, the large subject of the treatment of nocardia infections, in which glycopeptide resistance does not usually present a problem, will not be dealt with. As clinical experience of treatment is necessarily limited by the recent appearance of resistant strains, and assessment of response may be difficult in infections caused by these opportunistic gram-positive bacteria, information from studies in vitro and from biological models must be used.

Enterococcal Infections

It is not surprising that glycopeptide resistance should prove significant in enterococci, which intrinsically are susceptible to a narrow range of antibiotics and which have acquired significant, high-level resistance to the agents favored for treatment (258, 271). Indeed, the multiresistant enterococcus has been named the nosocomial pathogen of the 1990s (357). From colonization of the gastrointestinal tract, the urethra, vagina, and mouth (153), infection may occur in the urinary tract, peritoneum, surgical wounds, and other sites, and bacteremia is common. Enterococcal infection is particularly associated with prosthetic devices and other foreign materials, such as endovascular devices, joint prostheses, and the cannulas used in CAPD (152, 318). Enterococci account for 5 to 20% of cases of infective endocarditis, including community-acquired infection (252).

Enterococcal endocarditis provides a defined entity, with a significant mortality of approximately 20%, in which choice of a treatment regimen with bactericidal activity is critical for success (189) and for which biological models have been shown to have predictive value (169). Assessment of significance in other situations may be more difficult. Undoubtedly, serious infection with enterococci, including multiresistant strains, does occur (298), and enterococcal sepsis is associated with a high mortality (165, 318), but the contribution of the enterococcus may be in doubt (271). The infections may be polymicrobic and occur in a setting of host impairment and rapidly evolving illness and changing infections. Bacteremia may be persistent, even in the absence of an endovascular or other focus, but not clearly associated with a serious outcome. For example, in a recent series of 125 episodes of enterococcal bacteremia (152), death occurred in 17.6%, and in 8% the fatal outcome was considered to be directly related to the bacteremia. However, 38% of the bacteremias were polymicrobic and the outcome was unrelated to the appropriateness of antimicrobial treatment, empirical, definitive, or considered together. It is inevitable that effective treatment for endocarditis should dominate the discussion of alternative treatment for glycopeptide-resistant strains.

The standard treatment for enterococcal endocarditis has been a bactericidal and synergistic combination of a β-lactam antibiotic (ampicillin or benzylpenicillin) with an aminoglycoside (usually streptomycin or, more recently, gentamicin) (49, 189, 421). The alternative, in patients who are hypersensitive to the β-lactam antibiotic or when there is high-level resistance to it (MIC, >100 μg/ml), is a combination of a glycopeptide and an aminoglycoside; such combinations also show bactericidal synergy (111, 246) but have no advantage over the usual regimen in customarily susceptible strains and may be more nephrotoxic; also, experience with the glycopeptide combination is more limited (49). The cell wall-acting agents are not adequately bactericidal alone, and thus the relative susceptibility of enterococci to the aminoglycoside is critical for the achievement of the desired effect. While there has been a tendency to use such combinations for the treatment of serious enterococcal infections other than endocarditis, there is no evidence that

they give any benefit over single agent treatment, for example, in septicemia (150, 165).

Significant resistance problems other than resistance to glycopeptides. High-level gentamicin resistance (MIC, >2,000 μg/ml), which abrogates the bactericidal synergism of the combinations described above with all aminoglycosides except streptomycin (to which also many high-level gentamicin-resistant strains are resistant by a separate mechanism), has become increasingly common, especially in the United States, where the prevalence may be 70% or more. There has been relatively little experience of treating endocarditis caused by such aminoglycoside-resistant strains, but the best recommendation is the use of a very high dosage, as by continuous infusion, of ampicillin alone (104); this usage is confirmed in the rat model (106, 371). Successful treatment of enterococcal endocarditis was achieved with penicillin alone in a proportion of cases in the era before the standard combination treatment was established (139). *E. faecium* is intrinsically less susceptible to β-lactams than is *E. faecalis*. Vancomycin is generally less bactericidal than the β-lactams, and while teicoplanin is usually more active (106), the glycopeptides offer no advantage over ampicillin in these strains with isolated high-level gentamicin resistance. It has been noted that rat serum may exert a synergistic effect with glycopeptides against some enterococci and that this might enhance glycopeptide activity (141).

High-level resistance to penicillins is increasing in prevalence, mediated by β-lactamase (272) or by modification of PBPs (410). While resistance by β-lactamase may be overcome to some extent, in the rat model of *E. faecalis* endocarditis, by use of a β-lactamase inhibitor such as sulbactam or clavulanate (190, 197, 227, 370), most β-lactamase-producing enterococci are also resistant to high-level gentamicin (103). Strains of *E. faecium* resistant at high levels to all aminoglycosides and to β-lactams create a setting in which the use of glycopeptides becomes of critical significance; glycopeptide resistance is beginning to present serious problems, and new approaches to the use of the agents customarily employed and alternative treatment regimens are needed.

Peptide antibiotics. Although neither vancomycin nor teicoplanin alone is satisfactorily bactericidal to enterococci in vitro (282, 304, 361), showing less activity than is observed against staphylococci (160), both agents reduce the bacterial counts of susceptible enterococcal strains in the vegetations in animal endocarditis models and may eradicate the infection, teicoplanin generally achieving better results than vancomycin (111, 426). Against a strain of *E. gallinarum* (at first thought to be *E. faecium*) with low-level resistance to vancomycin (MIC, 16 μg/ml) but susceptible to teicoplanin (MIC, 1 μg/ml), both agents in moderate dosage caused a small reduction in vegetation counts, much smaller than that seen with a glycopeptide-susceptible variant of the strain. High-dose teicoplanin (20 mg/kg, giving mean peak and trough concentrations of 63 and 25 μg/ml, respectively), however, achieved results against the resistant parent similar to the results obtained against the susceptible variant with moderate dosage (116). Septicemia caused by *E. faecium* resistant to vancomycin but remaining susceptible to teicoplanin has been treated successfully with teicoplanin (122), and intrathecal teicoplanin (accompanied by systemic treatment with several other antibiotics) has been used successfully to treat postoperative meningitis caused by a similar strain of *E. faecium* (240).

There has been considerable effort to develop alternative agents in the same group, either as totally new compounds or as modifications of vancomycin or teicoplanin. Daptomycin (LY146032), an acidic lipopeptide, gave promising results in vitro. MICs were low in most investigations (8, 34, 39, 47, 77, 110, 257, 283, 348), with all strains of all species of *Enterococcus* inhibited by 8 μg/ml and MICs at which 90% of the strains are inhibited (MIC_{90}s) being recorded up to 4 μg/ml; glycopeptide-resistant *E. faecium* strains were included (47, 257). In other investigations (62, 204, 223) higher values were obtained, with MIC ranges and MIC_{90}s up to 100 μg/ml; glycopeptide-resistant enterococci were also included here (204). The difference is unexplained. The inoculum effect was small, the agent was effectively bactericidal alone (unlike teicoplanin and vancomycin) (110, 241), and there was no cross-resistance with vancomycin or teicoplanin. Addition of serum to the medium impaired the in vitro activity (46, 138, 233, 257, 360). Variable, but quite encouraging results were obtained in animal models, enterococcal pyelonephritis (254, 337), endocarditis (63, 310), and the hamster model of pseudomembranous colitis (94); in the last, daptomycin was more effective than vancomycin. Although daptomycin was well tolerated, the early results in clinical trials were disappointing (137), with, for example, failure to overcome staphylococcal septicemia. This may well have been the result of the very high (>90%) plasma protein binding (323), and high dosage might have succeeded (225, 323). Unfortunately, higher dosage regimens were associated with toxicity in humans, and the drug was withdrawn in 1990 (225).

Ramoplanin (A16686, MDL 62198), a lipoglycodepsipeptide, is even more active. In all investigations (34, 84, 204, 206, 228, 257, 293, 348, 425), all strains of all species, including vancomycin-resistant *E. faecalis* and *E. faecium* (84, 204, 257), were inhibited by 8 μg/ml, and the MIC_{90}s (in all but one study [34]), ranged from ≤0.25 to 1.6 μg/ml. Ramoplanin is also highly bactericidal for enterococci, with the MBC within four times the low MIC (204). Addition of 50% human serum gives a fourfold increase in MIC. Ramoplanin seems unsuitable for systemic use because of toxicity but is being developed for topical use. It has been suggested that ramoplanin could be used for the clearance of glycopeptide-resistant enterococci from the gastrointestinal tract (204) and that it might well be used to eradicate *C. difficile*, without risk of colonization by glycopeptide-resistant enterococci (43).

Other peptide antimicrobial agents have been investigated in vitro for activity against enterococci. Derivatives of vancomycin (286) may show increased activity against enterococci, including glycopeptide-resistant strains (286), but development of these compounds is at an early stage. Three derivatives of teicoplanin, MDL 62208, MDL 62211, and MDL 62873, are more active against enterococci than is the parent compound; for example, MDL 62873 was reported to have MIC ranges for all species of ≤0.12 to 2 μg/ml and MIC_{90}s of up to 0.65 μg/ml in three studies (30, 34, 62). Cross-resistance with vancomycin and teicoplanin-resistant strains, however, is reported (42). LY 264826 is more active than vancomycin and teicoplanin in tests of inhibitory activity (MIC_{90}, up to 0.5 μg/ml [62, 77]) but is not bactericidal against enterococci (77). Derivatives of LY264826 with greater activity against glycopeptide-resistant enterococci (VanA and VanB phenotypes) have been produced. For a series of VanA strains, the MICs were as follows: vancomycin, 64 to 2,108 μg/ml; LY264826, 2 to 64 μg/ml; derivative, 0.03 to 2 μg/ml. The derivatives also showed bactericidal activity and effectiveness in a mouse endocarditis model (285). Decaplanin, mersacidin (M87-1551), actaplanin, and SK&F 104662 do not have the advantage of consistently better in vitro activity than those of vancomycin and teicoplanin, or of increased bactericidal activity, and tend to show cross-resistance against glycopeptide-resistant strains (30, 283, 331, 353, 425).

It is clear that before this group of antimicrobial agents can offer new treatment possibilities for these infections further work is needed to produce a satisfactory bactericidal glycopep-

tide, effective and nontoxic in systemic use and lacking cross-resistance with vancomycin and teicoplanin (119).

Antibiotic combinations with glycopeptides. As stated above, high-level resistance to aminoglycosides abrogates synergism in the combination with β-lactams and glycopeptides. Resistance to these cell wall-active agents themselves has varying effects on this synergism, depending on the degree and mechanism of the resistance (125, 184). Resistance to β-lactams of moderate or even a high degree caused by modification of PBPs may still allow synergism to occur (65, 375), the exact mechanism of such resistance being variable among strains (222). Glycopeptide resistance itself does not usually affect β-lactam–aminoglycoside synergy in strains susceptible to the latter agents (47, 229). In general, if strains are not highly resistant to aminoglycosides, synergism can be shown with cell wall-active agents provided that sufficiently high concentrations of the latter drugs in relation to their MICs can be obtained (65, 375). Although in some cases the expected synergism has not been observed to occur, this may have been due to peculiarities of aminoglycoside transport, such as have been observed in the past (184, 259). Adequate anti-cell wall activity may be obtained with single drugs or with a combination of β-lactam and glycopeptide (67).

While with fully susceptible isolates the effect of a combination of ampicillin and vancomycin is generally antagonistic (103), the action of the combination on resistant enterococci varies. Some groups report synergism (47, 166, 229), which may even show a modest bactericidal effect in vitro or in animal models (65, 346), while others have not seen this phenomenon (71, 125, 157). It has been suggested that the action of the glycopeptide in this synergism may be the induction of modified peptidoglycan precursors which may have a low affinity for PBP5, so that other PBPs, such as PBP2 and PBP3 (for which the β-lactam has high affinity) become "essential" (5, 166, 167). The inconsistent results achieved may depend on differences in the mechanism of β-lactam resistance in the strains tested. These differences, as well as the quantitative degree of resistance to each agent (including the aminoglycoside) in individual strains, may also account for the inconsistent results obtained with the triple combination gentamicin-ampicillin-vancomycin, in which (in the absence of high-level gentamicin resistance) gentamicin might be expected to confer bactericidal synergy on the generally bacteriostatic synergism of the β-lactam–glycopeptide combination (125). Some investigators report striking bactericidal synergism in the triple combination (346), and this effect has also been shown in the rabbit model (65, 67), whereas others have achieved disappointing results, particularly with highly ampicillin-resistant and highly glycopeptide-resistant (VanA phenotype) strains (125).

To some extent these differences may be a function of the techniques used to demonstrate synergism in vitro. As expected, there may be discrepancies among double disk diffusion, checkerboard, and time-kill techniques. The striking bacteriostatic synergism that may be observed between vancomycin and an array of β-lactams, including cephalosporins, against glycopeptide-resistant enterococci by the first two methods is not usually accompanied by bactericidal activity (229). Observation of synergism in time-kill studies may depend on inoculum and the time of recording an endpoint (24 or 48 h) (184).

Although daptomycin alone shows good bactericidal activity against glycopeptide-resistant enterococci in vitro, this activity is enhanced by the addition of gentamicin (110, 229), provided, as always, that the strain does not show high-level gentamicin resistance. This effect was important in a rabbit endocarditis model in which the action of daptomycin alone was impaired (66). On the other hand, daptomycin and fosfomycin, which showed synergism in time-kill studies, gave no significant improvement over daptomycin alone in the rat endocarditis model (316). Ramoplanin with benzylpenicillin showed synergistic bactericidal activity in the rabbit model, when ramoplanin alone was ineffective (60). Vancomycin combined with ciprofloxacin showed synergism against a vancomycin- and ciprofloxacin-resistant enterococcus isolate but not at clinically achievable concentrations (381), and the demonstrated synergy in bacteriostatic terms between vancomycin and cephalosporins would not be adequate to indicate use of such combinations in their own right (350).

TABLE 6. Characteristic in vitro activities of quinolones against enterococci

Compound	Species	μg/ml			Reference
		MIC_{50}	MIC_{90}	MIC range	
Ciprofloxacin		1	4	0.24–4	
Sparfloxacin	*E. faecalis*	0.5	1	0.12–2	31
Sparfloxacin	*E. faecium*	1	1	0.5–1	31
Clinafloxacin	*E. faecalis*	0.125	0.125	≤0.007–2	255
Clinafloxacin	*E. faecium*	0.125	0.5	0.015–0.5	255
Tosufloxacin	*E. faecalis*	0.12	0.5	0.03–0.5	31
Tosufloxacin	*E. faecium*	0.5	1	0.12–1	31
Temafloxacin	Undefined	1	4	0.12–4	32
WIN 5727	*E. faecalis*	0.08	0.16	0.04–0.16	208
DU 6859	*E. faecalis*	0.2	0.39	0.1–1.56	338
DU 6859	*E. faecium*	0.39	1.56	0.05–6.25	338
OPC 17116	*E. faecalis*	0.2	0.39	0.1–0.78	400
OPC 17116	*E. faecium*	3.13	12.5	0.1–50	400
CP 99219	*E. faecalis*	0.25	0.5	0.25–0.5	106
CP 99219	*E. faecium*	2	4	0.25–8	106
PD 131628	*E. faecalis*	0.25	0.5	0.125–0.5	83

Fluoroquinolones. In the face of these unsatisfactory results, interest has turned to agents with other mechanisms of action (425), including the fluoroquinolones. Ciprofloxacin and other quinolones introduced during the same period (such as ofloxacin, norfloxacin, and enoxacin) have only modest activity against enterococci; a bactericidal effect is inoculum dependent and may be seen only at concentrations unattainable systemically in clinical use (36, 121, 270, 302). Effectively, their use is limited to the treatment of urinary tract infections (56, 271). Although ciprofloxacin in high, persistent concentrations may be effective in enterococcal endocarditis in the rat model (108), regimens closer to those usable in humans have not yielded satisfactory results (104, 121). Newer compounds with greater activity against gram-positive bacteria, however, have been created (302), and while enterococci remain among the least susceptible of gram-positive bacteria (with *E. faecium* in general being less susceptible than *E. faecalis*), some compounds inhibit 90% of strains at 1 μg/ml or less (302). Characteristic results of in vitro testing of several of these newly introduced quinolones are given in Table 6, which indicates that clinafloxacin (PD 127391, CI 960, and AM-1091) is the most active agent. Other compounds showing promise include PD 138312 and PD 140248 (193). Although strains of *E. faecalis* and *E. faecium* resistant to these newer quinolones are encountered (300, 400), several investigations have included strains resistant to other nonquinolone antimicrobial agents and obtained similar results (105, 208, 255, 289, 300, 425); in one case, gentamicin-resistant strains were significantly more resistant to the quinolone CP 99219 than gentamicin-susceptible strains (105). Temafloxacin has been withdrawn from

clinical development because of unexpected toxicity, and tosufloxacin is not being developed further (193).

Sparfloxacin and clinafloxacin have given promising results in vitro against glycopeptide-resistant *E. faecium* (61) and in the rabbit endocarditis model against *E. faecalis* and *E. faecium* (386). Clinical experience with the newer quinolones against glycopeptide-resistant strains is not reported. Potential problems are the comparatively poor bactericidal activity of some compounds, though not of all quinolones or against all strains of enterococci (289, 300, 302); the possibility of resistance emerging during treatment (302); and the effect of the trend that has been observed towards ciprofloxacin resistance among multiresistant enterococci (339), which may result in higher MICs and lack of bactericidal activity by the newer quinolones.

β-Lactam antibiotics. The conventional use of ampicillin, alone or in combination, or of benzylpenicillin in combination to treat enterococcal infections has been described above. For glycopeptide-resistant strains that are highly resistant to ampicillin or penicillin, no other β-lactam compound offers advantage. Ureidopenicillins are ineffective against such strains (187), and imipenem lacks bactericidal activity (22) and is not active against non-β-lactamase-producing, highly penicillin-resistant strains. Indeed, imipenem may act as a selective agent for enterococcal superinfection in hospital units (154). In addition, treatment of experimental endocarditis with imipenem has not given satisfactory results (22, 340). No cephalosporin has reliable activity alone, although some newer introductions, such as cefpirome (344), cefdinir (305), and RU 29246 (306), are more active than the earlier generations of cephalosporins.

Other agents. Rifampin alone has very limited usefulness in the treatment of enterococcal infections (260) because of its poor bactericidal activity (although it is capable of reducing bacterial counts in the vegetations of experimental endocarditis [404]) and because of the presence and emergence of subpopulations of resistant bacteria, both in vitro (260) and in vivo (404). As rifampin remains active against many multiresistant strains of enterococci, it is often tested in combination with other agents (see below). While the tetracyclines have bacteriostatic activity against enterococci, resistance is very common among hospital isolates resistant to favored antimicrobial agents (1). The glycylcyclines CL 329,998 and CL 331,002, *N*,*N*-dimethylglycylamido derivatives of minocycline and 6-demethyl-6-deoxytetracycline, respectively, show activity against most enterococcal strains, including those resistant to the parent drugs or to other antimicrobial agents. In a study including six species of *Enterococcus* and significant numbers of strains highly resistant to glycopeptides, β-lactams, and aminoglycosides, the highest MIC of CL 329,998 was 0.5 μg/ml (MIC_{50}, 0.06 to 0.25; MIC_{90}, 0.06 to 0.5) and that of CL 331,002 was also 0.5 μg/ml (MIC_{50}, 0.06 to 0.25; MIC_{90}, 0.12 to 1) (107). The activity remains bacteriostatic.

Quinupristin-dalfopristin (RP 59500, Synercid), a mixture of two water-soluble pristinamycins (modifications of pristinamycins I_A and II_A, respectively), may be given intravenously and has synergistic activity against gram-positive cocci, including enterococci (85, 118, 148, 395). The original mixture known as pristinamycin is also active (398) but is only available for oral administration. Numerical MIC results vary, but *E. faecium*, together with *E. durans* and *E. avium*, is generally more susceptible than *E. faecalis* (Table 7). Quinupristin-dalfopristin holds promise for treatment of infections caused by glycopeptide-resistant *E. faecium* strains, but some investigators have demonstrated less susceptibility in some glycopeptide-resistant strains than in glycopeptide-susceptible strains (85) (Table 7). Quinupristin-dalfopristin appears to be well tolerated (113). There are no reports of clinical trials in enterococcal infections, but accounts of successful treatment of individual patients have been published. Glycopeptide-resistant *E. faecium* was successfully eradicated from CAPD peritonitis, after intravenous and intraperitoneal administration, in three patients, in one case after retreatment with removal of the dialysis cannula (242). A central nervous system shunt infection caused by a vancomycin-resistant strain of *E. faecium* in a child was cured by intrathecal and intravenous quinupristin-dalfopristin (274). Apparent cure of *E. faecium* prosthetic valve endocarditis, after teicoplanin failure, has been briefly reported (135). Experience in treating nine patients (mainly recipients of solid organ transplants) suggested that quinupristin-dalfopristin is effective in preventing bacteremia but less so in dealing with focal infection by glycopeptide-resistant enterococci (235).

TABLE 7. In vitro activity of quinupristin-dalfopristin against enterococci

Species[a]	μg of quinupristin-dalfopristin per ml			Reference
	MIC_{50}	MIC_{90}	MIC range	
E. faecalis	4	4	1–32	118
E. faecalis	1	2	0.25–8	148
E. faecalis	4	4	1–8	395
E. faecalis, VS	16	32	2–32	85
E. faecalis, VR	32	32	4–32	85
E. faecium	1	2	0.25–4	118
E. faecium	1	4	0.25–8	148
E. faecium, VS	1	1	0.5–2	85
E. faecium, VR	1	16	0.5–32	85
E. avium	2	2	0.5–2	118
E. avium	2	8	0.25–8	148
E. durans	1	4	0.25–8	118

[a] VS, vancomycin susceptible; VR, vancomycin resistant.

Trospectomycin, an aminocyclitol and analog of spectinomycin with activity against a wide range of gram-positive bacteria, appeared promising in that in some investigations it showed uniform activity against *E. faecalis*, *E. faecium*, and other species (MIC, ≤32 μg/ml; MIC_{90}, 4 to 8 μg/ml) whether or not they were resistant to the available aminoglycosides or to ampicillin or vancomycin (219, 392). These results, however, were not confirmed in another investigation (401) which found a trospectomycin MIC_{90} of >64 μg/ml for various *Enterococcus* spp. It is acknowledged that trospectomycin alone has no significant bactericidal activity (219, 256, 392), and the combination of trospectomycin with ampicillin (or vancomycin) has not given the hoped for bactericidal synergy (219, 392). In a time-kill curve investigation of 11 strains of *E. faecium* (256), there was bacteriostatic synergy between ampicillin and trospectomycin against seven strains, but a modest bactericidal effect could be seen in only one-third of the strains. The usefulness of this effect in vivo has not been established.

Fosfomycin has activity against enterococci (172), but rapid emergence of resistance militates against its use alone. Trimethoprim, alone or in combination with a sulfonamide, displays bacteriostatic activity against enterococci (377), and trimethoprim-sulfamethoxazole is ineffective in experimental endocarditis (155) and also in a mouse peritonitis model (76). It may be used to treat urinary tract infection, although its effectiveness against enterococci in vivo has been disputed (173, 258).

Enterococci are marginally susceptible to macrolides such as erythromycin, and high-level resistance is becoming common; resistance to clindamycin is also implied (macrolide-lincosamide-streptogramin β phenotype) (271). Many *E. faecalis* strains show borderline or moderate resistance to chloram-

phenicol (MIC_{90}, 12.5 to 25 μg/ml) (373). In one investigation, resistance to chloramphenicol was found in 40% of such isolates (1). These antibiotics contribute little to the management of infection by glycopeptide-resistant enterococci.

Nitrofurantoin may also be effective in urinary tract infection (271). MICs of clofazimine were within attainable serum concentrations for eight of nine *E. faecalis* strains, but the activity was usually bacteriostatic (385). Sodium fusidate may inhibit multiresistant *E. faecium* but is not bactericidal. Novobiocin has been shown to be consistently active in vitro (MIC, 0.5 to 1 μg/ml) against vancomycin- and ampicillin-resistant *E. faecium* and to achieve a bactericidal effect against the majority of strains; *E. faecalis* was significantly more resistant (131). High plasma protein binding and problems of administration and toxicity limit the systemic use of novobiocin, although it might be serviceable for topical use. The nonabsorbable antibiotic bacitracin may be active against glycopeptide-resistant enterococci and has been used in initial studies to eradicate intestinal carriage (292). This function has also been suggested as a possible use for ramoplanin (see above).

Glycopeptide-resistant *E. faecium* isolates showed normal susceptibility to the disinfectant chlorhexidine (23), and aqueous preparations of chlorhexidine or povidone-iodine produced a rapid (≤3-min) ≥4-$\log_{10}$ fall in bacterial count (with occasional survivors) in a hand disinfection model (399). In one investigation, 15 strains of glycopeptide- and penicillin-resistant *E. faecium* were all susceptible to mupirocin, which was bacteriostatic (377), but another study demonstrated at least moderate degrees of resistance (MIC_{90} for *E. faecalis* strains, ≥16 μg/ml [206]).

Other combinations. Usually combinations of antimicrobial agents do not give improved results over those with the more active agent alone, and again, apparently contradictory results have been obtained by different investigators. The effect of a combination may depend on strain differences, including the quantitative level of resistance to each component. Most success with combinations has been obtained with agents that are sometimes bactericidal to enterococci when acting alone, such as ciprofloxacin and rifampin.

Ciprofloxacin was itself bactericidal (in a pharmacokinetic in vitro model) to a vancomycin-resistant (but aminoglycoside-susceptible) strain of *E. faecalis*, for which the MIC of ciprofloxacin (1 μg/ml) was relatively low; the addition of netilmicin or teicoplanin slightly increased the effect (36). Ciprofloxacin was not bactericidal (in killing curves) to a group of 15 glycopeptide- and β-lactam-resistant *E. faecium* strains. Nevertheless, if the ciprofloxacin MIC for these strains was ≤8 μg/ml, the addition of ampicillin produced a synergistic, bactericidal effect. Ciprofloxacin combined with novobiocin has also been shown to be bactericidal to strains relatively susceptible to ciprofloxacin (131, 226), and this effect has been demonstrated in the rabbit endocarditis model (307). Addition of ampicillin to clinafloxacin improved the bactericidal activity against glycopeptide-resistant *E. faecium* in vitro (shown against 12 of 12, rather than 7 of 12, strains) and prevented regrowth (61). There is one report of success in a neutropenic model in vivo with ciprofloxacin and azlocillin combined (301). In other reports, ciprofloxacin–β-lactam combinations have been disappointing (121, 262, 327).

In general, combinations of other agents with rifampin give an indifferent effect, the activity of the rifampin component (whether in killing curves or in animal models) depending on the quantitative susceptibility of the particular strains; in borderline rifampin susceptibility, the addition of another agent, such as trimethoprim-sulfamethoxazole, may marginally increase activity (377). Against one strain of glycopeptide-resistant *E. faecium*, which was relatively susceptible to the three agents ciprofloxacin, rifampin, and gentamicin, rifampin was effective in reducing bacterial counts in rat endocarditis vegetations, and all combinations of these antibiotics, including the triple combination, were synergistically bactericidal (404). One important function of combination treatment may be a reduction in the resistant subpopulations that may emerge, particularly in the case of rifampin (404).

Very little has been published concerning treatment with combinations that include newer antibiotics with activity against enterococci, although it is inevitable that they will be tested when this therapeutic dilemma presents itself. There is one report, for example, of eradication, in a neutropenic patient, of septicemia caused by a glycopeptide-, aminoglycoside-, and β-lactam-resistant strain of *E. faecium* with the combination of amoxicillin (high dosage), trimethoprim-sulfamethoxazole, and pristinamycin after trimethoprim-sulfamethoxazole and pristinamycin together had failed (234).

Conclusion. In severe enterococcal infections, such as endocarditis and septicemia in a compromised patient, the conventional regimen of penicillin or glycopeptide plus aminoglycoside is by far the most reliably effective treatment and should be invoked if at all possible. The effect can usually be predicted in strains with customary susceptibility to the agents concerned. In multiresistant strains, if the degree of resistance to one or more favored agents is moderate, it may nevertheless be worthwhile to test combinations, including the penicillin-glycopeptide-aminoglycoside combination, in killing curves (using high but attainable serum concentrations). For highly aminoglycoside-resistant strains, long-continued treatment with sustained high levels of a cell wall-active agent offers the best hope of success; again, the penicillin-glycopeptide combination may be tested against multiresistant strains. While these and other unusual antibiotics are tested in vitro, other forms of treatment, such as early valve surgery, should be considered (103).

Staphylococcal Infections

Glycopeptide resistance among *Staphylococcus* spp. presents therapeutic problems quite different from those seen with resistance in enterococci in that clinically threatening degrees of resistance to glycopeptides have not been observed in the most pathogenic species, *S. aureus* (maximum MIC, 8 μg/ml), and severe degrees of resistance to vancomycin have been observed uncommonly in any species. Resistance to teicoplanin in CNS, particularly *S. haemolyticus* and *S. epidermidis*, has been the main phenomenon at issue (26, 142, 194).

The clinical significance of glycopeptide resistance has been disputed, and the difficulty is compounded by the variation in the results in vitro, according to the conditions employed in testing. The earliest reports of resistance to vancomycin in *S. epidermidis* may have been influenced by the large inoculum used in the susceptibility testing (343, 349, 380). The unsatisfactory results with disk testing of susceptibility to teicoplanin may create difficulty in recognition of comparatively resistant strains. This has particular importance in units that use teicoplanin empirically as a matter of policy. The NCCLS, in a review of clinical data and in vitro results, apparently had no problems (205) in adopting breakpoints for teicoplanin MICs (≥32 μg/ml = resistant; ≤8 μg/ml = susceptible [279]), but others have concluded that in vitro susceptibility test results are poorly predictive of outcome (75, 79, 291). Dosage regimens have been revised upwards since the early, unsuccessful, clinical trials of teicoplanin (64, 136); in particular, higher than standard dosage (or the use of combinations of antimicrobial agents) is required for treatment of septic arthritis and of *S.*

aureus endocarditis (412), although higher dosage may be associated with toxic effects (237, 412).

Undoubtedly, glycopeptide treatment failures have occurred with development of resistance or superinfection by resistant strains (21, 57, 212, 334, 343, 387), and substitution of alternative agents has sometimes proved successful (21, 57, 343). There is a tendency for more resistant strains in general to be isolated from patients who have received or are receiving teicoplanin, and units which use teicoplanin extensively may yield isolates with higher modal MICs than are generally observed (251).

CNS showing resistance in vitro to teicoplanin or vancomycin tend to be resistant to other antistaphylococcal agents; for example, in one series of clinical isolates, 70% of isolates of *S. epidermidis* that showed moderate resistance to teicoplanin were resistant to oxacillin (26). When teicoplanin resistance is detected, or when there is clinical failure after teicoplanin administration, vancomycin may usually be substituted (26, 57, 251). In endocarditis, the infection in which CNS have the most clearly defined pathogenic role, the standard recommendation is use of an isoxazolyl penicillin, such as oxacillin or flucloxacillin, replaced by vancomycin in β-lactam-resistant strains, with addition of rifampin and/or gentamicin when the infection is associated with prosthetic material (49, 216), according to the susceptibility of the strain. There is no indication to vary these recommendations in the case of teicoplanin-resistant CNS that have remained susceptible to other agents, and there is thus often an acceptable alternative treatment.

Testing of individual isolates should indicate the possibilities for alternative treatment. Treatment failures with glycopeptide-resistant staphylococci have responded to treatment with tobramycin (administered intraperitoneally for CAPD peritonitis [334]) and to a combination of sodium fusidate and rifampin (21), as well as to vancomycin, in the case of teicoplanin failure (57). An anxiety is the possibility of vancomycin resistance appearing in MRSA, which is often resistant to most other possible agents; such an event would make urgent the search for alternative regimens.

Among other peptide antibiotics, daptomycin is uniformly active against staphylococci, including strains for which teicoplanin MICs are up to 8 μg/ml, with all strains tested inhibited by 4 μg/ml and 90% inhibited by 2 μg/ml and with no significant difference according to susceptibility to oxacillin and related penicillins (39, 77, 223, 283, 348); slightly higher MICs for *S. hominis* were recorded in one series (8). Ramoplanin has similar or greater activity (127, 228, 348). In a collection of over 1,000 staphylococci, mostly identified to species level, the MIC_{50} of ramoplanin was 0.5 μg/ml and the MIC_{90} was 1 μg/ml. For only 17 isolates of CNS (from 11 patients) were ramoplanin MICs >2 μg/ml, and the highest MIC was 8 μg/ml (in three strains, two of *S. simulans* and one of *S. haemolyticus*). MICs of ramoplanin and teicoplanin were correlated (127). Other new glycopeptides, SK&F 104662, LY264826, and decaplanin, had MICs for CNS similar to, or greater than, those of teicoplanin (77, 283, 331, 353), but some derivatives of teicoplanin appear more active than the parent compound (42). The limitations of the use of daptomycin and ramoplanin discussed in relation to enterococci make necessary further development within this group before the newer glycopeptides can become useful in the treatment of systemic infections by antibiotic-resistant staphylococci.

On the other hand, developments among the fluoroquinolones appear more immediately promising. Ciprofloxacin and the others of that "generation" are more effective against staphylococci than they are against enterococci, and the newer introductions (discussed above for enterococci) are even more active, so that in vitro 90% of strains are inhibited by 0.25 μg/ml or less (302). In particular, the bactericidal action against staphylococci (68), the long serum half-lives, and encouraging results in the animal endocarditis model (210, 321) indicate that fluoroquinolones may be a challenger to the admittedly not entirely satisfactory, standard regimens for infective endocarditis, providing oral and well-tolerated treatment (74, 101). On the other hand, development of resistance to ciprofloxacin or fleroxacin during treatment, experimental or clinical, even in combination use (209, 211, 367), and the increasing occurrence of resistant strains in units where fluoroquinolone usage is high (25, 295) may limit this application; the activity of newer quinolones with greater activity against staphylococci may be impaired against ciprofloxacin-resistant strains, and resistance may be more likely to emerge (213).

Quinupristin-dalfopristin is very active and bactericidal (191) against staphylococci, 90% of *S. aureus*, *S. epidermidis*, *S. haemolyticus*, and *S. hominis* strains, whether or not resistant to oxacillin and related penicillins, being inhibited by ≤1 μg/ml (3, 118, 148, 191, 395). Success in experimental staphylococcal (including MRSA) endocarditis has been demonstrated (112, 117). The influence of erythromycin resistance on results in vivo depends on the degree of susceptibility to the quinupristin component (117). As this compound may be pursued for the treatment of infections by MRSA and by enterococci, it may have an occasional role in the future against infection by CNS (11).

Infections by Other Glycopeptide-Resistant Bacteria

The largest series of glycopeptide-resistant gram-positive bacteria of other species systematically examined for susceptibility to antimicrobial agents included 79 clinical isolates (43 *Leuconostoc*, 13 *Lactobacillus*, and 23 *Pediococcus* spp.) among 85 strains (364). All strains were resistant to vancomycin and teicoplanin, and almost all showed moderate resistance to benzylpenicillin (MIC range, 0.03 to 2 μg/ml; MIC_{90}, 1 μg/ml) and ampicillin; activity of the cephalosporins was variable, but all *Lactobacillus* and *Pediococcus* spp. and the majority of *Leuconostoc* strains were susceptible to imipenem. All strains were susceptible to gentamicin, and almost all were susceptible to erythromycin, clindamycin, and chloramphenicol. Variable results were obtained for the other antimicrobial agents tested. It seems that there is no currently available antimicrobial agent to which susceptibility can be assumed: there are reports of tetracycline, clindamycin, and gentamicin resistance in *Leuconostoc* spp. (40, 159, 176) and resistance to all β-lactams, aminoglycosides, macrolides, tetracyclines, and quinolones in *Pediococcus* spp. (250, 366, 425). Penicillin tolerance appears to be a feature of *Leuconostoc* spp. (176, 198). Individual clinical isolates require careful testing when they are considered to be of pathogenic significance.

All strains in the major investigation (364) were susceptible to the withdrawn peptide antibiotic daptomycin (MIC range, ≤0.25 to 0.5 μg/ml; MIC_{90}, 0.25 μg/ml), and this observation has been confirmed by others (90, 144, 366). Ramoplanin also was effective against a collection of 14 *Leuconostoc* spp., 23 *Lactobacillus* spp., 3 *Pediococcus* spp., and 9 unidentified gram-positive cocci, all resistant to vancomycin but all inhibited by ≤0.5 μg of ramoplanin per ml (84). Mersacidin showed variable activity against a small collection of vancomycin-resistant species; for the *Leuconostoc* spp., MICs were ≤2 μg/ml, but for the four *Pediococcus* spp., a range of MICs was seen: 4 to 32 μg/ml (30).

The pristinamycin mixture quinupristin-dalfopristin (RP59000) was similarly effective in vitro, inhibiting all strains (35 *Lacto-*

bacillus spp., 24 *Leuconostoc* spp., 9 *Pediococcus* spp., 9 unidentified vancomycin-resistant gram-positive bacilli, and 2 *Erysipelothrix* spp.) at ≤2 μg/ml (85).

Although *Leuconostoc* and *Pediococcus* spp. undoubtedly may be pathogenic in causing signs of infection (40, 144), it is rare to be able to identify any contribution towards mortality in the patient, and bacteremia may resolve without treatment (176, 249). Among 23 cases of *Leuconostoc* infection (of blood or cerebrospinal fluid) in the literature (40, 133, 176), 4 were said to have died (although the role of *Leuconostoc* spp. was doubtful), while 18 were cured or improved; in one case further information was not available. Six of the patients with a favorable outcome received no specific treatment or had the intravascular cannula (which was the defined or assumed portal of entry for the bacterium) removed, while nine received benzylpenicillin (in one case, with gentamicin), two received clindamycin, and one received nafcillin. Penicillin has been recommended as the treatment of choice pending laboratory investigation (40).

In the case of isolation of *Pediococcus* spp., their contribution to disease may be even more obscure (249). In many cases of isolation from blood, the bacteremia has been polymicrobic (249, 351). Although patients with *Pediococcus* bacteremia have received a variety of antimicrobial agents subsequently, particularly β-lactam agents alone or with an aminoglycoside, it is difficult to discern any consistent pattern of response.

Tolerance to penicillins is characteristic of *Lactobacillus* strains isolated from cases of endocarditis, and combination with an aminoglycoside may overcome this. These in vitro results are reflected in experience with patients in whom relapse may follow treatment with a single agent, and eventual cure may take place with the combination (37, 38, 88, 162), although failures have been reported and large doses of penicillin and an aminoglycoside may be required (362, 368). Nevertheless, the combination of penicillin and an aminoglycoside may be the initial treatment of choice for endocarditis caused by unusual glycopeptide-resistant gram-positive cocci while definitive testing is carried out.

Endocarditis caused by a vancomycin-resistant *Corynebacterium* sp. has been successfully treated with imipenem and ciprofloxacin (28). CAPD peritonitis caused by *Lactobacillus casei* responded slowly to intraperitoneal erythromycin and oral rifampin (332).

Erysipelothrix rhusiopathiae infections are customarily treated with benzylpenicillin (145) or with cephalosporins, and these (with imipenem) proved to be the most active agents in a study of 10 *E. rhusiopathiae* strains (391) (MIC ranges, penicillin, ≤0.01 to 0.06 μg/ml; cefotaxime, ≤0.01 to 0.12 μg/ml; imipenem, ≤0.01 to 0.06 μg/ml), while susceptibility to ciprofloxacin was also noted (MIC range, ≤0.01 to 0.25 μg/ml). Variable results were obtained with erythromycin, clindamycin, tetracycline, and chloramphenicol. Blood isolation of *E. rhusiopathiae* is usually, but not always (376), associated with endocarditis (145). Long courses of treatment with benzylpenicillin alone (145) or the conventional treatment for viridans streptococcal endocarditis, with benzylpenicillin plus gentamicin (151), has usually been employed; a case in which this regimen failed but cure was produced by penicillin combined with high-dose ciprofloxacin has been reported (243).

CONCLUDING REMARKS

The last 5 years have seen major advances in our understanding of the mechanism of glycopeptide resistance, particularly in enterococci. However, the origin of both the resistance genes and the resistant bacteria themselves has yet to be determined. In particular, we still need to know whether glycopeptide-resistant enterococci evolve specifically in hospitals or whether they are imported from the community and are then given a selective advantage by widespread antimicrobial agent use in the hospital setting. An understanding of this is critical, as once established, the prevention and control of hospital infections and colonizations by glycopeptide-resistant enterococci are problematic. A document highlighting this specific area was recently published by the Hospital Infection Control Practices Advisory Committee (192). Multiresistance in glycopeptide-resistant bacteria poses major therapeutic problems, and the need for alternative treatment regimens is becoming urgent. Currently, the most promising fields of development appear to be those of the developmental peptides and quinolones. However, the future may see the emergence of new problems as well as solutions to current ones. Perhaps the most worrying possibility is the specter of glycopeptide resistance arising in, or spreading to, MRSA.

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