The development of resistance to glycopeptides (vancomycin and teicoplanin) is one of the best examples of the alternation between success and failure that spans the history of the antibiotic era. The introduction of penicillin G was revolutionary. However, the emergence in the 1950s of clinical isolates of Staphylococcus aureus that were resistant to penicillins and subsequently to erythromycin and tetracyclines stimulated the development of large-scale screening programs for new drugs.

Under these circumstances, an American missionary, Reverend W. M. Bouw, provided Eli Lilly (Indianapolis) with a soil sample from Borneo [1]. The organism that produces vancomycin, Amycolatopsis orientalis, was isolated from this sample in 1954. Since vancomycin proved to be potent against gram-positive bacteria, including penicillinase-producing staphylococci, it was licensed for human use in record time. However, the renal toxicity and ototoxicity associated with vancomycin therapy (due for the most part to impurities in the drug) together with the introduction of new penicillinase-resistant β-lactams led to a rapid decline in the use of vancomycin.

In the 1980s, interest in vancomycin revived because of several factors. During this period, methicillin-resistant and multiresistant staphylococci, against which vancomycin is often the only active antibiotic, were emerging worldwide. At the same time, medical advances had resulted in the prolongation of life for increasingly fragile, immunocompromised patients who are exposed to opportunistic pathogens such as multiresistant gram-positive organisms. In addition, it was determined that pseudomembranous colitis could be treated with oral vancomycin, and a new glycopeptide, teicoplanin, was introduced in 1984 in certain European countries.

The apparent absence of resistance to vancomycin in gram-positive bacteria and a decrease in the toxicity associated with vancomycin therapy (purified formulations of the drug had become available) led to a notable increase in the prescription of this antibiotic. In the United States, a 20-fold increase in the consumption of vancomycin was observed over a 10-year period at a university hospital [2].

The emergence in 1986 of transferable glycopeptide resistance in enterococci [3, 4] was unexpected and had several consequences: (1) glycopeptide resistance in enterococci that were already resistant to other antibiotics resulted in severe clinical problems in certain countries; (2) possible dissemination of resistance to other pathogens, such as methicillin-resistant Staphylococcus aureus, became a concern; (3) it became difficult to control resistant infections because the multiple mechanisms by which resistance is disseminated in the community and the hospital had not (and still have not) been fully elucidated; and (4) isolation of glycopeptide-resistant enterococci from humans and animals in European communities suggested that there were dangers associated with the use of antibiotics (including a glycopeptide, avoparcin) for nonmedical purposes such as additives in animal feed. Finally, the biochemical mechanism and regulation of expression of acquired resistance to glycopeptides are one of the most sophisticated, perfect examples of the genetic adaptation of bacteria.

### Mechanism of Acquired Resistance to Glycopeptides

Glycopeptides are inhibitors of cell wall synthesis. In contrast to β-lactams, glycopeptides do not interact with cell wall biosynthetic enzymes. These drugs are large, rigid, hydrophobic molecules that form complexes with the peptidyl-d-alanyl-d-alanine termini of peptidoglycan precursors at the cell surface. The presence of these large complexes at the surface of the cytoplasmic membrane prevents cell wall synthesis by hindering the transfer of cytoplasmic precursors to the growing peptidoglycan chain and by blocking formation of interpeptidic bonds (figure 1) [5].

Resistance to glycopeptides is due to synthesis of modified precursors that display decreased affinity for vancomycin and teicoplanin (figure 1). This modification, which implies alteration of the peptidoglycan biosynthetic pathway, can result only from bacterial acquisition of a gene cluster encoding a coherent enzymatic machinery. VANA resistance is the type of resistance that has been most thoroughly studied [6]. In Enterococcus faecium strain BM4147, the resistance gene is carried by the 10.8-kb transposable element Tn1546 [7]. The transposon Tn1546 encodes seven polypeptides that act cooperatively to confer high-level vancomycin resistance [7]. Two of these polypeptides (VanR and VanS) are involved in the regulation of resistance-gene expression, three (VanH, VanA,
and VanX) confer resistance to glycopeptides, and two (VanY and VanZ) are accessory proteins that are not essential for the expression of glycopeptide resistance. Coordinated expression of VanH, VanA, and VanX is necessary to confer glycopeptide resistance.

Characterization of VanA provided the first clue as to the mechanism of resistance. VanA is homologous to bacterial ligases, which are chromosomally encoded enzymes that are involved in an early stage of the synthesis of the cell wall precursors. They synthesize a dipeptide D-alanyl-D-alanine (D-Ala-D-Ala), which is then branched to a tripeptidic precursor that is translocated at the cell surface. Vancomycin (V) binds to the D-Ala-D-Ala terminus of the pentapeptide chain and prevents subsequent steps in synthesis of the peptidoglycan. Right: the VanH dehydrogenase of a glycopeptide-resistant enterococcus synthesizes D-lactate from pyruvate. The broad-substrate-range VanA ligase catalyzes the formation of the depsipeptide D-Ala-D-Lac, which is then branched to UDP-N-acetyl muramyl tripeptide to form a pentadepsipeptide precursor. Vancomycin has a greatly reduced affinity for the precursors ending in D-Ala-D-Lac. As a consequence, enterococci can grow in the presence of glycopeptides. VanX and VanY are carboxypeptidases that prevent synthesis of the D-Ala-D-Ala dipeptide and the pentapeptide ending in D-Ala-D-Ala, respectively.

Cross-linking of the precursors to the growing peptidoglycan is processed in bacteria by the penicillin-binding proteins (PBPs), namely PBP5 in *E. faecium* [10]. The substitution of D-Ala by D-Lac does not impair cross-linking of the modified precursors to the growing peptidoglycan chain. However, PBPs other than PBP5, which are so far not known to have a role in cell wall synthesis, are probably required for processing of the altered precursors [11]. These high-molecular-weight PBPs display a higher affinity for β-lactams. Since VANA resistance is inducible, the shift in PBPs occurs only in the presence of vancomycin and results in β-lactam hypersusceptibility. This effect explains the synergy displayed by the combination of the two classes of drugs against vancomycin-resistant strains. The possible therapeutic implications of this synergy will be discussed below.

On the basis of the mechanism detailed above, it would appear that production of VanH and VanA is sufficient to confer glycopeptide resistance; however, this is not the case because, as already mentioned, the additional VanX protein is required. In fact, the chromosomal enzymes that produce the usual susceptible cell wall precursors are still functional in the vancomycin-resistant enterococci, which should be considered as merodiploids for cell wall synthesis. Depsipeptides and dipeptides are coproduced and compete for the synthesis of modified and unmodified precursors.
Enterococcal Resistance to Glycopeptides

The levels of vancomycin resistance depend on the respective proportions of each type of precursor [12]. Resistance will be phenotypically expressed only if the levels of precursors susceptible to vancomycin are sufficiently low. VanX is a D,D-di-peptidase that hydrolyzes D-Ala-D-Ala but not D-Ala-D-Lac and thus prevents synthesis of precursors ending in d-alanine [13]. The accessory protein VanY probably acts as a complementary lock: VanY is a D,D-carboxypeptidase that could contribute to resistance by cleaving the terminal D-Ala of late peptidoglycan precursors resulting from incorporation of D-Ala-D-Ala that escaped hydrolysis by VanX [14]. Finally, VanZ confers low-level resistance to teicoplanin by an unknown mechanism [15].

Diversity of Phenotypes and Genotypes for Acquired and Intrinsic Vancomycin Resistance

Acquired enterococcal resistance to glycopeptides was first detected in 1986 [3, 4]. Three phenotypes—VANA, VANB, and VAND—can be distinguished on the basis of the antibacterial and resistance-inducing activity of vancomycin and teicoplanin (table 1). The VANA phenotype is characterized by inducible resistance to high levels of vancomycin (MIC, \(\geq 64\) mg/L) and teicoplanin (MIC, \(\geq 16\) mg/L) [3, 6]. This class of resistance is usually mediated by self-transferable plasmids that bear transposons that are structurally closely related to Tn1546. The vanA genes were recently found on a mobile element, Tn5482 (composed of Tn1546 and insertion sequences IS1251), that could direct its own transfer from the chromosome of one Enterococcus strain to that of another strain [16].

VANB strains are inducibly resistant to various amounts of vancomycin (MICs range from 4 mg/L to \(\geq 1,000\) mg/L), but they remain susceptible to teicoplanin [17, 18] because vancomycin acts as an inducer whereas teicoplanin does not. Low-level and high-level VANB resistance is transferable by conjugation in certain strains [18, 19]. Transfer of resistance is associated with transfer of large (i.e., from 90 kb to 250 kb) genetic elements from chromosome to chromosome [18]. The 250-kb element has recently been characterized [20]; it contains the composite transposon Tn1547, which is flanked by insertion sequences related to IS236.

VAND is a recently described phenotype [21] characterized by resistance to moderate levels of vancomycin and low-level resistance or susceptibility to teicoplanin.

The vanA and vanB clusters encode related dehydrogenases (VanH and VanHb), ligases (VanA and VanB), and D,D-di-peptidases (VanX and VanXb), which is consistent with the fact that they confer resistance to glycopeptides by means of similar mechanisms [22].

As discussed above, the resistance genotype can be inferred from the phenotype characterized for vancomycin and teicoplanin. However, two unusual modes of expression of the VANA and VANB types of resistance deserve further description. First, clinical isolates of vancomycin-dependent VANB Enterococcus faecium have been reported [23–25]. These organisms require vancomycin for growth. It has been hypothesized that the chromosomal D-Ala-D-Ala ligase is not produced or is impaired in these strains and that expression of the VanB D-Ala-D-Lac ligase that can be induced by vancomycin allows for cell wall synthesis and survival of the strains in the presence of vancomycin [26, 27].

The exact incidence of vancomycin-dependent strains is unknown; however, in a rabbit model of aortic endocarditis due to a VANB strain, vancomycin-dependent mutants could easily be selected by treatment with vancomycin or teicoplanin [28]. VANA-type dependent mutants could also be obtained in vitro [26, 27]. The importance of vancomycin-dependent strains is due to the fact that they can be missed when a standard agar medium is used to isolate them.

In addition, a clinical isolate of VANB Enterococcus faecium that expressed cross-resistance to teicoplanin and vancomycin has been reported [29]. The strain was a constitutive mutant selected by vancomycin treatment in a patient infected with a VANB-inducible strain [29]. Similar VANB mutants can be selected by administration of teicoplanin in an animal model [28].

Strains belonging to Enterococcus gallinarum, Enterococcus casseliflavus, and Enterococcus flavescens are intrinsically re-

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**Table 1. Phenotypes of glycopeptide-resistant enterococci.**

<table>
<thead>
<tr>
<th>Phenotype, species</th>
<th>MIC (mg/L)</th>
<th>Transferable resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vancomycin</td>
<td>Teicoplanin</td>
</tr>
<tr>
<td>Acquired resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VANA*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. faecium</td>
<td>64–1,000</td>
<td>16–512</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>4–1,024</td>
<td>0.25–2</td>
</tr>
<tr>
<td>E. avium</td>
<td>16–64</td>
<td>2–4</td>
</tr>
<tr>
<td>E. durans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. hirae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. munditii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. raffinosus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. gallinarum</td>
<td>2–32</td>
<td>0.12–2</td>
</tr>
<tr>
<td>E. casseliflavus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. flavecens</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. NT = not tested.
* Also detected in Arcanobacterium haemolyticum and Cellulomonas turbata.
† Also detected in Streptococcus bovis.
sistant to vancomycin (MICs, 4–32 mg/L) but remain susceptible to teicoplanin; these organisms display the VANC phenotype (table 1). *E. gallinarum* and *E. casseliflavus-E. flavescent* (these organisms are thought to belong to the same species) contain the vanC1 and the vanC2 gene, respectively, which participates in the synthesis of peptidoglycan precursors ending in D-Ala-D-serine displaying reduced affinity for vancomycin [22, 30, 31]. Neither these bacteria nor other gram-positive organisms that are intrinsically resistant to glycopeptides (i.e., *Lactobacillus* species, *Pediococcus* species, and *Leuconostoc* species) appear to be the source of the genes encoding acquired resistance in enterococci [32].

**Dissemination of Glycopeptide Resistance: Facts and Thoughts**

Resistance to glycopeptides has spread primarily in *E. faecium* strains that are already resistant to a broad range of antimicrobial agents. Strains of *E. faecium* that are resistant to every antibiotic that can be used for efficient treatment of systemic infection (i.e., aminoglycosides, penicillins, and glycopeptides) have been described [33]. However, glycopeptide resistance is not confined to a single species, and the *vanA* gene cluster has been detected in a variety of enterococcal species (table 1).

A high incidence of glycopeptide resistance (mainly VANA resistance in *E. faecium*) is observed in certain countries and certain hospitals. For example, a 20-fold increase in the incidence of such resistance in the United States from 1989 (0.4%) to 1995 (10%) was reported by the Centers for Disease Control and Prevention [34]. Although vancomycin resistance was first reported in the United Kingdom and France, the incidence remains low in Europe—generally <2% [35, 36]. On the other hand, epidemics have been reported in certain hospitals, particularly in the United Kingdom [37].

The transfer of vancomycin resistance from enterococci to other gram-positive bacteria was predictable because there is no barrier against expression of the resistance genes in various gram-positive organisms including *Streptococcus* species, *Listeria monocytogenes*, and, of most importance, *S. aureus* [38, 39]. Corynebacterium-related bacteria (*Arcanobacterium haemolyticum* and *Cellulomonas turbata*) harboring *vanA*-related genes were recently isolated from the stools of some patients in the United Kingdom, and a strain of *Streptococcus bovis* harboring a *vanB*-related gene cluster was isolated from a patient in France [40, 41]; these findings represent an in vivo demonstration of this transferability. Conjugative transfer of glycopeptide resistance from *E. faecalis* to *S. aureus* has been reported only under laboratory conditions; this transfer has been observed both in vitro and in vivo on the skin of mice [39].

**Risk Factors for Colonization or Infection with Glycopeptide-Resistant Enterococci**

The risk factors for colonization or infection by glycopeptide-resistant enterococci have been investigated in numerous studies. There are often multiple factors involved; the role of a predominant factor is difficult to elucidate. Studies based or multivariate analysis are most suited to answer this question. But samples of a sufficient size are required. The results of several case-control studies have been published that establish the typical profile of a patient at risk for colonization or infection with glycopeptide-resistant enterococci (table 2). Such patients have received previous antibiotic treatment, mainly with third-generation cephalosporins (or other *β*-lactams); have severe underlying conditions (e.g., they have undergone liver or bone-marrow transplantation or have renal failure, cancer, diabetes, or decubitus ulcers); are hospitalized in a renal unit, oncology (including hematology) unit, intensive care unit, or surgical unit; have been hospitalized for prolonged periods; have been treated with multiple antibiotics including vancomycin and/or cephalosporins; and have undergone invasive procedures.

Most of the reported risk factors for colonization or infection are nonspecific, since they are the same for colonization or infection with vancomycin-susceptible enterococci [48]. Data from case-control studies in which nosocomial glycopeptide-resistant enterococci and glycopeptide-susceptible enterococci have been isolated from patients are shown in table 2. These studies again showed that treatment with vancomycin or third-generation cephalosporins as well as length of hospital stay were independent risk factors.

In one study, administration of metronidazole was a significant risk factor for the development of bacteremia due to glycopeptide-resistant enterococci [43]. The authors of this study hypothesized that the elimination of anaerobes from the gut might confer an advantage to enterococci in terms of colonization that subsequently leads to bacteremia. Administration of other antibiotics, such as moxalactam, clindamycin, or imipenem, with activity against anaerobes has also been found to be a risk factor for colonization or infection due to enterococci or ampicillin-resistant enterococci [49–51]. However, these antibiotics have poor activity against enterococci and could also directly select for these microorganisms. In two studies, prior vancomycin therapy was associated with glycopeptide-resistant enterococcal bacteremia [47, 52].

**Clinical Experience**

The mortality associated with bloodstream infections due to enterococci, regardless of vancomycin resistance, is generally high—from 34% to 46%; however, enterococci are paradoxically considered to be weak pathogens [53]. This high mortality is usually attributed to the underlying conditions of the patients, which predispose to the development of enterococcal bacteremia. The clinical outcome for patients infected with glycopeptide-resistant enterococci is similar overall, with mortality rates of >50%, although these rates have varied from 8% to 73% in recent studies [43, 45–47, 54–57].
Enterococcal Resistance to Glycopeptides

Table 2. Summary of data from case-control studies of patients infected with glycopeptide-resistant enterococci.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Ward</th>
<th>Source of glycopeptide-resistant enterococi (no. of cases)</th>
<th>Glycopeptide resistance phenotype (no. of strains)</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>[42]</td>
<td>Univariate analysis</td>
<td>Medical-surgical/ICU</td>
<td>Blood (4), urine (2), stools (3)</td>
<td>VANA, Enterococcus faecium (9)</td>
<td>Duration of ceftazidime treatment, no. of days in ICU (no isolation)</td>
</tr>
<tr>
<td>[43]</td>
<td>Univariate analysis</td>
<td>Oncology</td>
<td>Blood (11)</td>
<td>VANA, E. faecium (11)</td>
<td>Intestinal colonization with glycopeptide-resistant enterococci, use of antibiotics active against anaerobes</td>
</tr>
<tr>
<td>[44]</td>
<td>Univariate analysis</td>
<td>Various*</td>
<td>Various (colonization + infection) (41)</td>
<td>VANA, E. faecium (6), VANB, E. faecium (35)</td>
<td>Previous exposure to antibiotics, use of third-generation cephalosporins, use of parenteral vancomycin</td>
</tr>
<tr>
<td>[45]</td>
<td>Multivariate analysis</td>
<td>Various†</td>
<td>Various (20)</td>
<td>NA</td>
<td>Use of multiple antibiotics (ciprofloxacin, aztreonam, vancomycin), severity of illness</td>
</tr>
<tr>
<td>[46]</td>
<td>Multivariate analysis</td>
<td>Various (mainly oncology-hematology, AIDS, surgical, ICU)</td>
<td>Blood (46)</td>
<td>E. faecium (40, including 38 VANA), Enterococcus faecalis (2)</td>
<td>Hematologic malignancy, use of vancomycin, severity of illness†</td>
</tr>
<tr>
<td>[37]</td>
<td>Multivariate analysis</td>
<td>Hepatology (included transplant recipients)</td>
<td>Various (colonization + infection) (38)</td>
<td>NA</td>
<td>Length of hospital stay</td>
</tr>
<tr>
<td>[47]</td>
<td>Multivariate analysis</td>
<td>Liver transplantation</td>
<td>Blood (54)</td>
<td>VANA (54)</td>
<td>Length of hospital stay</td>
</tr>
</tbody>
</table>

NOTE. ICU = intensive care unit; NA = data not available.
* Seven hospitals, including both primary and tertiary care centers.
† One hospital.
‡ Based on APACHE II score.

Glycopeptide-resistant enterococci seem to more often cause life-threatening diseases than do their glycopeptide-susceptible counterparts [43, 54], although one study did not show any difference [57]. For instance, Linden et al. [47] conducted a case-control study that showed that vancomycin resistance played a critical role in the recurrence of bacteremia and in the associated mortality; shock and liver failure were other cofactors. Virulence factors specific to glycopeptide-resistant enterococci have not been identified so far. In fact, the high mortality rates could be due to the fact that the patients who have developed bloodstream infections with such strains have been severely ill and therefore have been at higher risk of dying. Indeed, a careful study of mortality due to enterococcal bacteremia showed that if glycopeptide-resistant enterococci are more likely to cause death (15 of 46 patients infected with resistant strains died) than are glycopeptide-susceptible enterococci (3 of 46 patients infected with susceptible strains died), the difference was no longer significant after controlling for severity of illness and gender [46]. Another study showed that immunocompromised patients are more likely to have bloodstream infections than are immunocompetent patients [55].

The lack of uniformly effective therapy for patients infected with glycopeptide-resistant enterococci is also an important consideration. Although the results with respect to the role of appropriate therapy in reducing the mortality associated with enterococcal infections have conflicted, Hoge et al. [58] showed that if the clinical significance of bacteremia is strictly defined, specific therapy is associated with improved outcome.

Therapeutic Options

Penicillin G, ampicillin, or amoxicillin combined with an aminoglycoside remains the therapy of choice for infections due to glycopeptide-resistant enterococci. For E. faecium infections, only gentamicin (or streptomycin) should be used in combination with a β-lactam, since the remaining aminoglycosides are inactivated by a species-specific chromosomal acetylating enzyme (AAC(6')-I) and do not display synergism with β-lactams [59, 60]. However, most glycopeptide-resistant E. faecium express resistance to one or both classes of antibiotics.

As mentioned above, combinations of vancomycin and various β-lactams have been shown in vitro to have bacteriostatic synergy but not bactericidal synergy against glycopeptide-resistant enterococci [61, 62]. However, mutants resistant to these
synergistic combinations can readily be observed both in vitro and in animal models [62, 63], and synergism is not observed for strains that are highly resistant to penicillins [64].

Finally, the clinical effectiveness of combined therapy is not known. In one study, the addition of gentamicin to the β-lactam—vancomycin combination prevented the emergence of mutant strains in animals, provided that the strain was not highly resistant to the aminoglycoside [63]. The combination of ampicillin and imipenem appeared efficient in an experimental model of endocarditis in rabbits [65].

Chloramphenicol, fluoroquinolones, tetracycline, and rifampin have been used against susceptible enterococci, but these antibiotics are not bactericidal [56, 66]. A limited clinical study showed that chloramphenicol was only partially efficient [56]. In an experimental model of endocarditis in rats, a 5-day course of treatment with ciprofloxacin/rifampin/gentamicin was efficient, although selection of rifampin-resistant mutants occurred in all animals [67].

Novobiocin and bacitracin have been used in attempts to suppress fecal carriage of glycopeptide-resistant enterococci, but these drugs have had limited effects [68, 69]. Quinupristin/dalfopristin is an injectable streptogramin that has been used in a compassionate protocol and is under investigation [70]. Several other antimicrobial agents are at an early stage of development, but their usefulness has not yet been proven.

Prevention: the Reservoirs and Routes of Dissemination

Since there is no fully efficient therapy for infections due to glycopeptide-resistant enterococci, efforts to limit the spread of these microorganisms are now considered essential. Prevention is first based on the knowledge of the reservoirs and the routes of dissemination of glycopeptide-resistant enterococci.

Hospital-associated infections due to glycopeptide-resistant enterococci can derive endogenously from the patient's own flora, or such infections can be exogenously acquired via the spread of an epidemic strain, or both. Several European studies have demonstrated that glycopeptide-resistant enterococci are part of the normal human fecal flora. These bacteria have been found outside hospitals, in waste waters [71–73], and in the feces of nonhospitalized patients [71, 74, 75] including healthy volunteers who were not health care workers, had never received glycopeptides, and had not been treated with antibiotics during the year preceding the study [76].

Incidence of 1.3% and 28% (Belgium) [75, 76], 2% (France) [77], and 12% (Germany) [74] have been reported for community-acquired infections, which contrast with the low incidence of infections in hospitals (see above). The wide variations in the incidences could be explained in part by differences in the sensitivity of the stool culture methods that were used.

A possible source of glycopeptide-resistant enterococci is the food chain, since VANA E. faecium has been isolated from farm animals in the United Kingdom [72], Germany [78], and Denmark [79] and from animal-derived food products, including frozen poultry [72, 78]. German and Danish investigators have raised the possibility that the glycopeptide avoparcin, used for nearly 20 years as a feed additive in animal husbandry in numerous European countries (but not the United States or Canada), could have selected for glycopeptide-resistant enterococci in the digestive tracts of animals. It has been observed that the VANA strains are cross-resistant to vancomycin, teicoplanin, and avoparcin, a finding consistent with this possibility.

VANA E. faecium has been isolated from pigs and chickens grown on German and Danish farms where avoparcin is used, whereas the enterococci isolated from the feces of animals raised on farms that do not use avoparcin are susceptible to vancomycin [74, 78, 79]. The enterococci isolated from animals raised on the Danish farms contained clusters of vanA gene closely related to that found in enterococci isolated from humans [80]. However, other growth promoters among the numerous antibiotics used might have selected glycopeptide-resistant enterococci because these organisms were resistant to several antibiotics (i.e., macrolides, tetracyclines, and aminoglycosides).

In the United States, attention has focused on the epidemiology of glycopeptide-resistant enterococci, mainly in hospitals rather than in the community, since glycopeptide-resistant enterococci have most often been responsible for outbreaks of infection in the intensive care units of large hospitals. In a study in Texas, investigators failed to find any glycopeptide-resistant enterococci in the feces or carcasses of chickens [81]. In addition, glycopeptide-resistant enterococci could not be isolated from healthy volunteers from the community in two studies [45, 81].

In hospitals in the United States, most outbreaks of glycopeptide-resistant enterococci are due to intrahospital or interhospital spread of clonal strains [52, 82], although unrelated bacteria can also be isolated [45, 82]. This circumstance suggests that patient-to-patient transmission is the major factor responsible for dissemination of glycopeptide-resistant enterococci in the United States.

The reasons for the differences between the European and North American rates of resistance are still unknown. Hypothetically, these differences could be related to differences in the use of glycopeptides, resulting in distinct antibiotic selective pressures specific to individual countries. For example, in Europe, oral administration of avoparcin could have favored intestinal carriage of glycopeptide-resistant enterococci outside hospitals, initially in animals and then in humans, via the food chain, whereas in North America, heavy use of both intravenous and oral vancomycin in hospitals could have led to selection of resistant enterococci primarily in clinical settings.

In any case, the intestinal reservoir is of major importance, and isolation of glycopeptide-resistant enterococci from clinical samples correlates with carrier status [57]. Glycopeptide-resistant enterococci have been isolated from the hands of health care workers [46]. However, the role of environmental contamination in the transmission of these
organisms has not been fully elucidated and has probably been underestimated so far.

The results of surveillance screening during outbreaks have indicated that glycopeptide-resistant enterococci can be isolated from the inanimate environment in the wards where colonized or infected patients are located. Air cushions, automated medication dispensers, bed rails, blood pressure cuffs, control panels of pulse oximeters, doors, electrocardiographic monitors, electronic thermometers, glucose meters, headboards, linens, monitoring devices, overbed tables, stethoscopes, toilet surfaces, and ventilator tubing were found to be contaminated during recent outbreaks [37, 42, 43, 45, 46, 83]. Enterococci can survive for prolonged periods, both on the hands of health care workers and on environmental surfaces [84].

Prevention and Control of Infections Due to Glycopeptide-Resistant Enterococci

Of the risk factors for acquisition of colonization or infection by glycopeptide-resistant enterococci, antibiotic consumption and duration of treatment are amenable to intervention. The administration of vancomycin is frequently reported as a risk factor [44–46, 84, 85]. It is noteworthy that when oral vancomycin or teicoplanin was administered to 22 healthy Belgian volunteers, selection of large numbers of glycopeptide-resistant enterococci in the fecal flora of 14 of these volunteers was observed [76]. Similar selection of high counts of glycopeptide-resistant enterococci was reported when oral vancomycin was administered in an animal model [86].

Therefore, recommendations have been formulated by the Hospital Infection Control Practices Advisory Committee (HICPAC) that discourage the use of oral or parenteral vancomycin in many situations—for instance, for routine prophylaxis, for primary treatment of antibiotic-associated colitis, and for selective decontamination of the digestive tract (for details see [87]). Oral metronidazole can be used in place of vancomycin to treat colitis due to Clostridium difficile.

The impact of reducing the consumption of glycopeptides remains to be evaluated. Morris et al. [45] reported that in an attempt to control the spread of glycopeptide-resistant enterococci, reducing the consumption of parenteral and oral vancomycin by 59% and 85%, respectively, in combination with hygienic measures, did not result in changes in the rates of stool colonization and infection due to these strains. However, it must be stressed that at least no increase in the rates was observed and that the control study was done shortly after the implementation of an infection control program and thus should be repeated later. Clinicians should also remember that the use of third-generation cephalosporins is a common risk factor for infection due to glycopeptide-resistant enterococci and that prolonged therapy with these antibiotics should probably be avoided when possible.

Microbiology laboratories play a crucial role in the detection and timely reporting of colonization or infection due to vancomycin-resistant enterococci. It is particularly important to detect the initial isolates before colonization becomes endemic and then difficult to control. For early detection, laboratories should use reliable conventional broth and agar methods rather than automated systems, which perform poorly in detecting glycopeptide-resistant enterococci [88]. In addition, a multiplex PCR technique has been proposed for rapid detection and characterization of glycopeptide resistance genes [89].

If a patient is found to be infected or colonized with multi-resistant enterococci, fecal samples should be obtained to determine colonization status. If glycopeptide-resistant enterococci are detected, the next step is to limit the nosocomial spread of these organisms by instituting conventional rigorous infection-control measures. These nonspecific measures include the use of barrier precautions and isolation or grouping of the infected or colonized patients.

The barrier precautions should be strict and should include the use of gloves, masks, and gowns by persons who have contact with patients who are colonized or infected with glycopeptide-resistant enterococci. The importance of handwashing with an antiseptic (chlohexidine) soap after patient contact must be stressed. The optimal duration of isolation for carriers of glycopeptide-resistant enterococci has not been established. Asymptomatic carriage can last for several months [90], and glycopeptide resistant enterococci can be present in barely detectable numbers in the feces and can thus be missed by screening procedures [76].

The above measures have proved to be efficacious in controlling outbreaks of glycopeptide-resistant enterococci [91]. However, the relative efficiency of each measure remains to be evaluated. In one report, an outbreak could be controlled only when gowns were used in addition to gloves and the patients were placed in isolation [83]. In contrast, another study showed that the use of gowns in addition to gloves was no better than the use of gloves alone for controlling outbreaks of glycopeptide-resistant enterococci [92]. Barrier precautions alone might not be sufficient if multiple modes of reintroducing the organisms into hospitals (i.e., via the food chain or via the admission of healthy carriers from the community) exist.

The usefulness of stool culture surveys remains to be studied. The HICPAC recommended that periodic culture surveys be performed for critically ill patients in hospitals where such patients are numerous [87]. However, Wells et al. [57] showed that during one outbreak, stool cultures were negative for nearly 50% of patients who acquired glycopeptide-resistant enterococci.

Conclusion

Glycopeptide-resistant enterococci have emerged at different rates in Europe and North America. Although first reported in Europe [3, 4], resistant enterococcal strains have not disseminated extensively in European hospitals so far. However, investigation of the epidemiology of glycopeptide-resistant enterococci has not been fully elucidated and has probably been underestimated so far.
Enterococci has led investigators to question the use of antibiotics in animal feed and to stress the putative danger of this practice. By contrast, in the United States, rapid dissemination of resistance among enterococci led to investigations of the use of vancomycin in humans; the results of these investigations indicated that the routes by which dissemination of resistance occurs in the hospital ecosystem are more complex than initially thought.

References


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1. Resistance to vancomycin in enterococci is due to  
   A. production of a modified peptidoglycan precursor with diminished affinity for vancomycin.  
   B. overproduction of a modified peptidoglycan precursor with increased affinity for vancomycin, which traps the drug.  
   C. enzymatic modification of vancomycin.  
   D. impermeability to the drug.  

2. Which of the following statements concerning the VANA and VANB types of resistance is correct?  
   A. Expression of VANB resistance is usually constitutive, whereas expression of VANA resistance is inducible.  
   B. VANA resistance is potentially more dangerous than VANB resistance, since only VANA resistance is transferable.  
   C. The biochemical mechanisms of glycopeptide resistance in VANA and VANB strains are similar.  
   D. Both VANA and VANB strains are always highly resistant to vancomycin (MICs, >128 mg/L).  

3. The vanA or vanB gene cluster has not been detected in clinical isolates belonging to which of the following bacterial species?  
   A. Streptococcus bovis  
   B. Staphylococcus aureus  
   C. Cellulomonas turbata  
   D. Arcanobacterium haemolyticum  
   E. Enterococcus faecalis  

4. Fecal carriage of glycopeptide-resistant enterococci  
   A. is found only in hospitalized patients.  
   B. can last for several months.  
   C. is probably not favored by the administration of oral vancomycin, since this drug does not select for large numbers of resistant enterococci in the gut.  
   D. is not the main source of infection in patients.  

5. In the United States, the combination of ampicillin/gentamicin cannot be used in many instances for treatment of infections due to glycopeptide-resistant Enterococcus faecium for all but which of the following reasons?  
   A. The isolates are often highly resistant to penicillins.  
   B. The isolates are often highly resistant to gentamicin.  
   C. The isolates are often co-resistant to penicillins and gentamicin.  
   D. All E. faecium strains produce a chromosomal acetylase (AAC(6')-I) that inactivates gentamicin.  
   E. renal failure.  

6. All of the following items have been described as major risk factors for acquisition of glycopeptide-resistant enterococci except  
   A. previous treatment with third-generation cephalosporins.  
   B. treatment with vancomycin.  
   C. prolonged hospital stays.  
   D. upper respiratory tract infections.  
   E. renal failure.  

7. Which of the following drugs can be an alternative to vancomycin for treatment of colitis due to Clostridium difficile?  
   A. Gentamicin  
   B. Kanamycin  
   C. Clindamycin  
   D. Metronidazole  
   E. Colistin  

8. The increased mortality rate associated with infections due
to glycopeptide-resistant enterococci vs. that associated with glycopeptide-susceptible enterococci is probably due to the following factors except
A. the acquisition of virulence factors by the strains.
B. the severity of underlying illnesses in the infected patients.
C. the lack of effective therapy.
D. the high frequency of bloodstream infections.

9. Which of the following statements concerning the use of antibiotics for animal feed is false?
A. Avoparcin is used for feeding animals in Europe but not in the United States.
B. VANA enterococci are cross-resistant to avoparcin, teicoplanin, and vancomycin.
C. Feeding tetracycline to animals probably cannot select for glycopeptide-resistant enterococci, since most strains are susceptible to this drug.
D. German and Danish studies suggest that glycopeptide-resistant enterococci are more frequent in farms where avoparcin is used than in those where this antibiotic is not used in animal feed.

10. The following measures for preventing the spread of glycopeptide-resistant enterococci have been generally recommended except
A. restricted use of oral and parenteral vancomycin.
B. detection of vancomycin resistance by reliable techniques in microbiology laboratories.
C. selective digestive tract decontamination.
D. barrier precautions for infected or colonized patients.