

Vancomycin-Resistant Enterococci

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INTRODUCTION	686
MECHANISMS OF RESISTANCE	687
β-Lactam Resistance	687
Aminoglycoside Resistance	687
Vancomycin Resistance	687
Phenotypic description	687
Genotypic classification and resistance mechanisms	688
(i) Action of vancomycin on peptidoglycan synthesis	688
(ii) VanA glycopeptide resistance	688
(iii) VanB glycopeptide resistance	689
(iv) VanC glycopeptide resistance	689
(v) VanD glycopeptide resistance	689
(vi) VanE glycopeptide resistance	689
(vii) Other resistance classes and organisms	689
(viii) Vancomycin-dependent enterococci	690
EPIDEMIOLOGY AND CONTROL	690
Geographic Distribution and Spread within Hospitals	690
VRE in Long-Term-Care Facilities	690
VRE in the Community	690
Risk Factors	691
Colonization and Infection	692
Reservoirs	692
Modes of Transmission	693
Prevention and Control Measures	693
Prudent use of vancomycin	694
Education programs	695
Role of the microbiology laboratory in the detection, reporting, and control of VRE	695
Implementation of infection control measures	696
Control of VRE in long-term-care facilities	697
Surveillance cultures	698
Attempts to eradicate gastrointestinal colonization	698
TREATMENT	698
REFERENCES	702

INTRODUCTION

Enterococci were originally classified as enteric gram-positive cocci and later included in the genus *Streptococcus* (185). In the 1930s, with the establishment of the Lancefield serological typing system, enterococci were classified as group D streptococci and were differentiated from the nonenterococcal group D streptococci such as *Streptococcus bovis* by distinctive biochemical characteristics (149). Sherman further recommended that the term “enterococcus” should be used specifically for streptococci that grow at both 10 and 45°C, at pH 9.6, and in 6.5% NaCl and survive at 60°C for 30 min (232). These organisms were also noted to hydrolyze esculin in the presence of bile. In the 1980s, based on genetic differences, enterococci were removed from the genus *Streptococcus* and placed in their own genus, *Enterococcus* (228). The previously used species

designations such as *faecalis*, *faecium*, *durans*, and so forth were retained but were preceded by the genus name *Enterococcus* in place of *Streptococcus*. Although a dozen *Enterococcus* species have been identified, only two are responsible for the majority of human infections. Until recently, *E. faecalis* had been the predominant enterococcal species, accounting for 80 to 90% of all clinical isolates, and *E. faecium* had accounted for 5 to 15% (110, 158, 173, 204, 219). Other *Enterococcus* species (*E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. avium*, and *E. raffinosus*) are isolated much less frequently and account for less than 5% of clinical isolates (110, 158, 173, 204, 219).

Enterococci have been recognized as an important cause of endocarditis for almost a century. In addition to this long-established role, enterococci began to be recognized as common causes of hospital-acquired infections in the middle to late 1970s. This was coincident with and probably related to the increasing use of third-generation cephalosporins to which enterococci are naturally resistant (185). Enterococci are currently ascendant nosocomial pathogens, having become the second most common organisms recovered from nosocomial urinary tract and wound infections and the third most common

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TABLE 1. Intrinsic and acquired antimicrobial drug resistance in enterococci^a

Intrinsic resistance
β-Lactams (particularly cephalosporins and penicillinase-resistant penicillins)
Low concentrations of aminoglycosides
Clindamycin
Fluoroquinolones
Trimethoprim-sulfamethoxazole
Acquired resistance
High concentrations of β-lactams, through alteration of PBPs or production of β-lactamase
High concentrations of aminoglycosides
Glycopeptides (vancomycin, teicoplanin)
Tetracycline
Erythromycin
Fluoroquinolones
Rifampin
Chloramphenicol
Fusidic acid
Nitrofurantoin

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cause of nosocomial bacteremia in the United States (227). One of the major reasons why these organisms have survived in the hospital environment is their intrinsic resistance to several commonly used antibiotics and, perhaps more important, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons (Table 1) (53). Therapeutic difficulties presented by enterococci were well recognized as early as the 1950s, when response rates of enterococcal endocarditis to penicillin used alone were found to be markedly lower than those of streptococcal endocarditis (73, 77). Because most enterococci are tolerant to the bactericidal activity of β-lactam and glycopeptide antibiotics, bactericidal synergy between one of these antibiotics and an aminoglycoside is needed to treat most serious enterococcal infections such as endocarditis and meningitis (144, 175). Since the duration of therapy is longer and the toxicity of the combination regimens is increased compared to those used for streptococcal endocarditis, enterococci are considered problem organisms with respect to antimicrobial therapy (186). The synergistic bactericidal effect between aminoglycosides and β-lactam or glycopeptide antibiotics is lost if there is high-level resistance to either class of drug. Resistance to high concentrations of aminoglycoside antibiotics is usually mediated by aminoglycoside-modifying enzymes, and it is widespread among enterococci (more than 50% of isolates in some centers show this resistance) (267). Also, many isolates of *E. faecium* are highly resistant to penicillins, because their penicillin binding proteins (PBP) have low affinity for penicillins (113). Until recently, vancomycin was virtually the only drug that could be consistently relied on for the treatment of infections caused by multidrug-resistant enterococci.

Vancomycin had been in clinical use for more than 30 years without the emergence of marked resistance (129). Teicoplanin is another glycopeptide antibiotic; it is not available in the United States but has been used in Europe. Because of their activity against methicillin-resistant staphylococci and other gram-positive bacteria, these drugs have been widely used for therapy and prophylaxis against infections due to these organisms (109). Oral vancomycin, which is poorly absorbed, has

been used extensively for the treatment of *Clostridium difficile* enterocolitis.

In 1988, Uttley et al. were the first to report the isolation of vancomycin-resistant *E. faecalis* and *E. faecium* in England (248). Shortly after the first isolates of vancomycin-resistant enterococci (VRE) were reported by investigators in the United Kingdom and France (155, 248), similar strains were detected in hospitals located in the eastern half of the United States (104). Subsequently, VRE have spread with unanticipated rapidity and are now encountered by hospitals in most states (31, 43, 134).

MECHANISMS OF RESISTANCE

β-Lactam Resistance

Complete or relative resistance to β-lactams is a characteristic feature of the genus *Enterococcus*. *E. faecalis* is typically 10 to 100 times less susceptible to penicillin than are most streptococci, while *E. faecium* is at least 4 to 16 times less susceptible than *E. faecalis* (185). While most isolates of *E. faecalis* are inhibited by concentrations of penicillin or ampicillin (1 to 8 μg/ml) easily achievable in humans, isolates of *E. faecium* usually require an average of 16 to 64 μg/ml to inhibit growth, although some isolates are even more resistant (186). An additional problem with enterococci is that they are typically tolerant to β-lactams (i.e., MBC/MIC of >32). The major mechanism underlying this resistance has been the production of low-affinity PBP (95). Penicillin resistance is directly proportional to the amount of PBP5 (a specific PBP) produced (259). Fontana et al. showed that loss of the ability of a strain of *E. faecium* to produce PBP5 caused this highly penicillin-resistant strain to become hypersusceptible to penicillin (97). β-Lactamase-producing enterococci are infrequently isolated (182). Unlike most staphylococci, where β-lactamase production is inducible, β-lactamase production in enterococci is constitutive, low level, and inoculum dependent (127, 182).

Aminoglycoside Resistance

Early studies demonstrated that two types of streptomycin resistance occur in enterococci: (i) moderate-level resistance (MIC, 62 to 500 μg/ml), because of low permeability, which can be overcome with a penicillin (which increases the cellular uptake of the aminoglycoside); and (ii) high-level resistance (MIC, ≥2,000 μg/ml), which is either ribosomally mediated or due to the production of aminoglycoside-inactivating enzymes (171). Since enterococcal resistance to gentamicin and streptomycin occur by different mechanisms, it is important to test susceptibilities to both agents. Gentamicin resistance is predominantly the result of the presence of the inactivating enzyme 2"-phosphotransferase-6'-acetyltransferase conferring resistance to gentamicin, tobramycin, netilmicin, amikacin, and kanamycin. Hence, gentamicin resistance is a good predictor of resistance to other aminoglycosides except streptomycin. Streptomycin resistance is encountered mainly in enterococcal strains that produce streptomycin adenylyltransferase; these strains remain susceptible to gentamicin (127). Penicillin-aminoglycoside synergy does not occur in high-level aminoglycoside-resistant enterococci (streptomycin MIC, ≥2,000 μg/ml; gentamicin MIC, ≥500 μg/ml).

Vancomycin Resistance

Phenotypic description. There are five recognized phenotypes of vancomycin resistance, VanA, VanB, VanC, VanD, and VanE (7, 94, 206). Two of these (VanA and VanB) are

TABLE 2. Characteristics of phenotypes of glycopeptide-resistant enterococci^a

Characteristic	Phenotype				
	VanA	VanB	VanC	VanD	VanE
Vancomycin MIC ($\mu\text{g/ml}$)	64–>1,000	4–1,024	2–32	128	16
Teicoplanin MIC ($\mu\text{g/ml}$)	16–512	≤ 0.5	≤ 0.5	4	0.5
Most frequent enterococcal species	<i>E. faecium</i> , <i>E. faecalis</i>	<i>E. faecium</i> , <i>E. faecalis</i>	<i>E. gallinarum</i> , <i>E. casseliflavus</i> , <i>E. flavescens</i>	<i>E. faecium</i>	<i>E. faecalis</i>
Genetic determinant	Acquired	Acquired	Intrinsic	Acquired	Acquired
Transferable	Yes	Yes	No	No	No

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mediated by newly acquired gene clusters not previously found in enterococci. VanA and VanB resistance phenotypes were described primarily in *E. faecalis* and *E. faecium*. VanA-resistant strains possess inducible, high-level resistance to vancomycin (MICs, $\geq 64 \mu\text{g/ml}$) and teicoplanin (MICs, $\geq 16 \mu\text{g/ml}$) (Table 2) (7). Resistance can be induced by glycopeptides (vancomycin, teicoplanin, avoparcin, and ristocetin) and by nonglycopeptide agents such as bacitracin, polymyxin B, and robenidine, a drug used to treat coccidial infections in poultry (146). The details of vancomycin resistance have been best documented with the *vanA* gene cluster found on the transposon, or “jumping” genetic element, Tn1546 (7, 11). VanB isolates were initially believed to be inducibly resistant to more modest levels of vancomycin (MICs, 32 to $64 \mu\text{g/ml}$) but are susceptible to teicoplanin. It is now known that levels of vancomycin resistance among VanB isolates may range from 4 to $\geq 1,000 \mu\text{g/ml}$ whereas susceptibility to teicoplanin is retained. VanB resistance determinants also reside on large mobile elements that can be transferred from one strain of enterococcus to another (212, 213). The VanC resistance phenotype was described in *E. casseliflavus* and *E. gallinarum*, which demonstrate intrinsic, low-level resistance to vancomycin (MICs, 4 to $32 \mu\text{g/ml}$) and are susceptible to teicoplanin.

Certain limitations of this classification method have become evident over time. For example, the genetic determinants of the VanA phenotype have now appeared in *E. gallinarum* and other enterococcal species (68). In a strain of *E. avium*, the VanA resistance determinants conferred a typical level of resistance to teicoplanin but low-level resistance to vancomycin (MIC, $16 \mu\text{g/ml}$) (218). Additionally, mutants derived from VanB strains may exhibit resistance to teicoplanin and thus be phenotypically indistinguishable from VanA strains (126). Nevertheless, this phenotypic classification scheme is still useful, because it usually corresponds well to the genotypic classification and utilizes information that can be derived simply and inexpensively in a laboratory (80).

Genotypic classification and resistance mechanisms. (i) Action of vancomycin on peptidoglycan synthesis. Under normal conditions of peptidoglycan synthesis in enterococci, two molecules of D-alanine are joined by a ligase enzyme to form D-Ala–D-Ala, which is then added to UDP-N-acetylmuramyl-tripeptide to form the UDP-N-acetylmuramyl-pentapeptide. The UDP-N-acetylmuramyl-pentapeptide, when incorporated into the nascent peptidoglycan (transglycosylation), permits the formation of cross-bridges (transpeptidation) and contributes to the strength of the peptidoglycan layer (80). Vancomycin binds with high affinity to the D-Ala–D-Ala termini of the pentapeptide precursor units, blocking their addition to the growing peptidoglycan chain and preventing subsequent cross-linking (262, 265).

(ii) VanA glycopeptide resistance. The *vanA* gene and other genes involved in the regulation and expression of vancomycin

resistance (*vanR*, *vanS*, *vanH*, *vanX*, and *vanZ*) are located on a 10,581-bp transposon (Tn1546) of *E. faecium*, which often resides on a plasmid (11). Expression of these genes results in the synthesis of abnormal peptidoglycan precursors terminating in D-Ala–D-lactate instead of D-Ala–D-Ala. Vancomycin binds to D-Ala–D-Lac with markedly lower affinity than it does to the normal dipeptide product (37). The core protein functions favoring synthesis of pentadepsipeptide terminating in D-Ala–D-Lac are as follows. (i) VanA protein is a ligase of altered substrate specificity which produces D-Ala–D-Lac in preference to D-Ala–D-Ala (36). (ii) VanH protein is a D-hydroxy acid dehydrogenase which creates a pool of D-lactate for use in the above reaction (12). (iii) VanX protein is a D,D-dipeptidase lacking activity against D-Ala–D-Lac. This enzyme reduces pools of D-Ala–D-Ala produced by the native enterococcal ligase, thereby minimizing the competing synthesis of normal pentapeptide (215, 262).

VanA alone cannot confer resistance to vancomycin, probably because D-hydroxy acids such as D-Lac are neither natural products present in the environment of enterococci nor normally produced by enterococci (154). Thus, to synthesize D-lactate, enterococci must acquire the gene(s) within the *vanA* operon required to produce the substrate for VanA. VanH is responsible for the synthesis of D-lactate.

VanR and VanS proteins constitute a two-component regulatory system that regulates the transcription of the *vanHAX* gene cluster (8). VanS apparently functions as a sensor to detect the presence of vancomycin (7, 11) or, more likely, some early effect of vancomycin on cell wall synthesis (117). VanS then signals VanR, the response regulator, which results in activation, or turning on, of the synthesis of some other proteins (VanH, VanA, and VanX) involved in resistance. In VanA phenotype strains, either vancomycin or teicoplanin can induce the transcription, but the precise signals are still unknown (15). *vanY* and *vanZ* may contribute to but are not essential for resistance. VanY protein is a D,D-carboxypeptidase that cleaves the D-Ala terminal peptide from any normal peptide that may have been made, contributing modestly to resistance levels (53). VanZ modestly increases the MICs of teicoplanin but not of vancomycin, through mechanisms that have not yet been elucidated. It is not essential to the expression of the VanA phenotype.

Mapping of the *vanA* gene cluster from several U.S. isolates revealed some heterogeneity in organization (122, 123). Tn1546 existed intact in some strains but had insertion-like sequences between *vanS* and *vanH* in others. These vancomycin resistance gene clusters may be incorporated into even larger mobile elements containing additional insertion-like elements (122, 123).

Cross-linking of the precursors to the growing peptidoglycan is processed in bacteria by the PBPs, with PBP5 being used in enterococci (96). The replacement 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 play a role in cell wall synthesis, are probably required for processing of the altered precursors (6). These high-molecular-weight PBPs display a higher affinity for β -lactams. Since VanA resistance is inducible, a shift in the 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.

(iii) VanB glycopeptide resistance. VanB glycopeptide resistance in enterococci is mediated by an abnormal ligase (VanB) that is structurally related to VanA ligase (76% amino acid identity). VanB protein also favors the production of the pentadepsipeptide terminating in D-Ala-D-Lac (87). Genes analogous to their class A resistance counterparts are designated *vanH_B*, *vanX_B*, *vanY_B*, *vanR_B*, and *vanS_B* (15). Levels of D,D-dipectidase activity (VanX_B) correlate with levels of vancomycin resistance (10). There is a high degree of sequence identity (approximately 70%) between VanHAX and VanH_BXB_B but considerably less homology (25 to 35% sequence homology) between the RS and Y proteins of VanA and VanB VRE (10, 86). There is no gene counterpart of *vanZ* in these organisms. *vanY_B* is not found in all strains, and its position in the gene clusters differs from that of *vanY* in Tn1546 (9, 86). Recent reports have shown DNA sequence heterogeneity, suggesting three subtypes of the *vanB* ligase gene: *vanB-1*, *vanB-2*, and *vanB-3* (60, 203, 208). The regulatory system in class B strains appears insensitive to induction by teicoplanin (10, 53). Teicoplanin induces the synthesis of VanA-related proteins but does not induce the production of VanB-related proteins. On the other hand, vancomycin induces the synthesis of the resistance proteins of both systems, and in fact, if a teicoplanin-susceptible enterococcus with the *vanB* gene cluster is preexposed to vancomycin, the strain then tests teicoplanin resistant as well. In addition, teicoplanin-resistant mutants can be derived from teicoplanin-susceptible, *vanB*-containing enterococci when these organisms are plated onto teicoplanin-containing agar. Such mutants can also arise in vivo during therapy (186). Possible mechanisms for teicoplanin resistance of these mutants include the loss of their requirement for an inducer (that is, if they constitutively produce high levels of the vancomycin resistance proteins) and the ability of teicoplanin to act as an inducer.

Another difference between VanA- and VanB-type resistance is that VanA is more widely distributed. It is by far the predominant type of resistance reported in Europe. While VanB strains are fairly common in the United States, with some hospitals reporting VanB exclusively, VanA still predominates (51). The *vanA* ligase gene, has also been found in a wider range of enterococcal species as well as in *Corynebacterium* spp., *Arcanobacterium haemolyticum*, and *Lactococcus* spp., while *vanB* has been found primarily in *E. faecium* and *E. faecalis*. The difference in the dissemination of these resistance traits may be related to the observation that the *vanA* gene cluster is often located on a transposon similar to Tn1546, which, in turn, can be a part of a conjugative (transferable) plasmid (11, 119, 122, 123). Such a genetic arrangement is an excellent avenue for the dissemination of these genes. The *vanB* cluster is often located on the host chromosome and initially was thought not to be transferable to other bacteria. However, it can also occur on plasmids, and, even when it is chromosomal, this gene cluster has been transferable as part of large mobile elements, perhaps related to large conjugative transposons (212).

(iv) VanC glycopeptide resistance. Low-level resistance to vancomycin is typical of *E. gallinarum*, *E. casseliflavus*, and *E. flavescentis*. The nucleotide sequences of the *vanC-1* gene in *E. gallinarum*, the *vanC-2* gene in *E. casseliflavus*, and the *vanC-3* gene in *E. flavescentis* have been reported, although there is some disagreement about whether *E. flavescentis* is a legitimate enterococcal species (52). VanC ligase of *E. gallinarum* favors the production of a pentapeptide terminating in D-Ala-D-Ser. Substitution of D-Ser for D-Ala is presumed to weaken the binding of vancomycin to the novel pentapeptide. Insertional inactivation of *vanC-1* unmasks the concomitant production of the D-Ala-D-Ala pentapeptide in *E. gallinarum* (216). D,D-Dipectidase and D,D-carboxypeptidase activities analogous to those of VanA and VanB strains have been described. It is presumed that the level of resistance expressed represents the balance achieved between normal and abnormal peptidoglycan synthesis (80). The presence of variable amounts of D-Ala-D-Ala relative to D-Ala-D-Ser could account for the variable levels of vancomycin resistance observed among isolates of VRE carrying the VanC phenotype (186). That is, lower MICs could be explained by the presence of larger amounts of D-Ala-D-Ala, which enables vancomycin to inhibit cell wall synthesis, and higher MICs could be explained by a higher proportion of D-Ala-D-Ser. Resistance may be inducible or constitutive (221). The *vanC-2* gene of *E. casseliflavus* demonstrates approximately 66% nucleotide sequence similarity to *vanC-1*. Like *E. gallinarum*, these strains also possess an additional native ligase (189). There is extensive homology (98%) between the gene sequences of *vanC2* and *vanC3* (52). *vanA* genes have recently been identified in strains of *E. gallinarum* and *E. casseliflavus*, conferring higher levels of resistance to vancomycin (MIC, >256 μ g/ml) in these species than normally anticipated and also resulting in resistance to teicoplanin (68).

(v) VanD glycopeptide resistance. A novel vancomycin resistance gene designated *vanD* was first described in a New York Hospital in 1991 (206). The strain carrying this resistance trait was an *E. faecium* strain that was inhibited by vancomycin at 64 μ g/ml and teicoplanin at 4 μ g/ml. Partial sequencing of the ligase gene showed that it was distinct from but similar to the *vanA* and *vanB* ligase genes. Recently, three clinical isolates of vancomycin-resistant *E. faecium* carrying the VanD resistance trait were found in Boston (199), and the deduced amino acid sequence of VanD showed 67% identity to those of VanA and VanB. VanD appears to be located on the chromosome and is not transferable to other enterococci.

(vi) VanE glycopeptide resistance. The *vanE* vancomycin resistance gene has recently been described in *E. faecalis* BM4405, which is resistant to low levels of vancomycin (MIC, 16 μ g/ml) and susceptible to teicoplanin (MIC, 0.5 μ g/ml) (94). This new resistance phenotype has similarities to the intrinsic VanC type of resistance. The deduced amino acid sequence has a greater identity to VanC (55%) than to VanA (45%), VanB (43%), or VanD (44%) (94).

(vii) Other resistance classes and organisms. *Lactobacillus casei*, *Pediococcus pentosaceus*, and *Leuconostoc mesenteroides* are naturally resistant to glycopeptides. Although the terminus appears to be the same (D-Ala-D-Lac), as found in VRE with VanA or VanB phenotypes (24, 120), DNA from these organisms does not hybridize with resistance gene probes prepared from VRE (24, 120).

In the laboratory, conjugal transfer of VanA-type vancomycin resistance genes from enterococci to other gram-positive cocci has been accomplished. Recipient organisms in successful transfers have included group A and viridans group streptococci, *Listeria monocytogenes*, and *Staphylococcus aureus* (57, 189). Transfer of resistance genes to *S. aureus*, resulting in high

levels of resistance to vancomycin, was demonstrated both in vitro and on the skin of mice. This gives rise to concern that such transfer in humans under natural conditions indeed might be feasible (193). Vancomycin resistance genes have already been found in human isolates of nonenterococcal organisms. A *vanB*-related gene sequence (designated *vanB3*) has been found in *Streptococcus bovis* (208).

(viii) Vancomycin-dependent enterococci. An interesting phenomenon that has developed in some strains of VanA- and VanB-type VRE is that of vancomycin dependence (64, 261). These enterococci are not just resistant to vancomycin but now require it for growth. Vancomycin-dependent enterococci have been recovered from apparently culture-negative clinical samples by plating them onto vancomycin-containing agar, such as that used for isolation of *Campylobacter* or gonococci. A likely explanation for the phenomenon of vancomycin dependence is that these enterococci turn off their normal production of D-Ala-D-Ala and then can grow only if a substitute dipeptide-like structure is made. With most VanA- and VanB-type enterococci, this occurs only in the presence of vancomycin, which induces the synthesis of associated dehydrogenase (VanH) and ligase (VanA or VanB) that make D-Ala-D-Lac. The reason for the cell turning off the synthesis of D-Ala-D-Ala is that as long as vancomycin is present, D-Ala-D-Ala is not necessary for cell wall synthesis by VRE (186). Indeed, it is being destroyed by the action of VanX. Once the vancomycin is removed, D-Ala-D-Lac is no longer synthesized, and without either D-Ala-D-Ala or D-Ala-D-Lac, the cell cannot continue to grow or replicate. Reversion to vancomycin independence has been observed; it probably occurs by either a mutation that leads to constitutive production of D-Ala-D-Lac or one that restores the synthesis of D-Ala-D-Ala.

EPIDEMIOLOGY AND CONTROL

Geographic Distribution and Spread within Hospitals

Since their initial recovery from patients in the United Kingdom and France, VRE have been found in many other countries, including Australia, Belgium, Canada, Denmark, Germany, Italy, Malaysia, The Netherlands, Spain, Sweden, and the United States (261).

From 1989 through 1993, the percentage of nosocomial enterococcal infections reported to the Centers for Disease Control and Prevention's National Nosocomial Infections Surveillance system that were due to VRE increased from 0.3 to 7.9% (43). The increase was mainly due to the 34-fold rise (from 0.4 to 13.6%) of VRE infections in intensive care unit (ICU) patients, although a trend toward increased VRE infections also was noted in non-ICU patients (43).

Hospital outbreaks of infection or colonization have been reported with both VanA and VanB isolates (29). Such outbreaks may involve clonal dissemination of strains indistinguishable by pulsed-field gel electrophoresis (PFGE), not only within hospitals but also among several local hospitals (180). Multiple clones are often encountered, and sporadic isolates of unrelated strains may coexist with a predominant clone suspected of institutional spread (30, 147). In hospitals in which VRE outbreaks have been detected at an early stage, cases often have been caused by a single strain (29, 30, 51, 121, 164, 176). When VRE have been present in a hospital or community for months or years, molecular typing of isolates often reveals that vancomycin resistance has spread by plasmids or transposons to many different clones (51, 122, 167, 181, 235).

Patients may be colonized simultaneously with more than one strain of VRE (167, 252). Stool isolates of VRE have

included a number of different species such as *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, *E. avium*, and *E. mundtii* (19). Fortunately, rates of stool colonization with VRE among hospitalized patients far exceed infection rates with these organisms (147, 176). Gastrointestinal tract colonization with VRE may persist for weeks or months, and single negative cultures may be intermixed with positive cultures over time (176). During outbreaks, environmental cultures in hospital rooms have yielded VRE (29, 167, 235).

VRE in Long-Term-Care Facilities

The role of long-term-care facilities (LTCFs) in the epidemiology of VRE has not been well defined. In a study performed in Chicago, where VRE have been endemic for several years, it was found that 47% of patients admitted to a hospital from LTCFs were colonized with VRE (M. L. Elizaga, D. Beezhold, M. K. Hayden, et al., Abstract, Clin. Infect. Dis. 23:925, 1996). The prevalence of VRE among LTCF residents, however, varies considerably by geographic area (32). Several preliminary reports emphasized that 10 to 16% of patients admitted to the hospital from nursing homes were colonized with VRE (M. P. Revuelta, J. A. Nord, R. L. Yarrish, et al., Abstract, Clin. Infect. Dis. 21:730, 1995; S. J. Sargent, V. S. Baselski, L. D. Reed, et al., Abstract, Clin. Infect. Dis. 21:729, 1995). In two reports, most or all nursing-home residents who were colonized with VRE had been hospitalized in an acute-care hospital within the preceding 3 months (29; J. Quale, K. Patel, M. Zaman, et al., Abstract, Clin. Infect. Dis. 21:733, 1995). Bonilla et al. reported that VRE colonization of residents in a Veterans' Affairs (VA) LTCF in Michigan ranged from 9% (in December 1994) to 19% (in January 1996) (27). During the same periods, 47 and 33% of residents' rooms were contaminated with VRE, respectively. Transmission between roommates was not observed. During the surveys, it was found that 26 to 41% of health care workers carried VRE on their hands. In another recent study in a VA LTCF in Pittsburgh, vancomycin-resistant *E. faecium* was identified in 24 of the 36 patients at the time of transfer from an acute-care facility (35). VRE in these patients persisted for a median of 67 days after identification. Treatment of VRE colonization with antimicrobials prolonged colonization. Serial surveillance of the 34-bed ward showed that the rates of colonization were stable, with only three documented instances of VRE acquisition. The authors of this report also noted that during 2.5 years of surveillance for infection, a single case of bacteremia occurred in a patient in whom colonization with VRE could not be demonstrated by rectal swab culture and no infections occurred in patients colonized with VRE. These studies indicate that colonized residents of LTCFs may serve as a reservoir for VRE for acute-care hospitals, just as patients from acute-care hospitals may reintroduce VRE to an LTCF continually.

VRE in the Community

In the United States, attention has focused on the epidemiology of VRE mainly in hospitals, and there is little evidence to suggest that transmission of VRE to healthy adults occurs to any significant extent in the community (B. E. Murray, Editorial response, Clin. Infect. Dis. 20:1134-1136, 1995). In a study in Texas, investigators failed to find any VRE in the feces or carcasses of chickens (186; Murray, Editorial response). In addition, VRE could not be isolated from healthy volunteers in two studies (186, 252; Murray, Editorial response). Two cases of apparent community-acquired VRE urinary tract infections in New York City have been reported (104). In another case, the husband caregiver of an elderly woman colonized with

TABLE 3. Summary of data from case-control studies in patients infected with VRE^a

Reference	Ward type	Source of VRE isolates (no. of cases)	Resistance phenotype and species (no.)	Statistical analysis used	Risk factors
164	Medical-surgical/ICU	Blood (4), urine (2), stools (3)	VanA, <i>E. faecium</i> (9)	Univariate	Duration of ceftazidime treatment, no. of days in ICU
69	Oncology	Blood (11)	VanA, <i>E. faecium</i> (11)	Univariate	Intestinal colonization with VRE, use of antibiotics active against anaerobes
180	Various ^b	Various (colonization + infection) (41)	VanA, <i>E. faecium</i> (6)	Univariate	Previous exposure to antibiotics, use of third-generation cephalosporins, use of parenteral vancomycin
181	Various	Various (20)	VanB, <i>E. faecium</i> (35)	Multivariate	Use of multiple antibiotics (ciprofloxacin, aztreonam, vancomycin), severity of illness
230	Various (mainly oncology-hematology, AIDS, surgical, ICU)	Blood (46)	<i>E. faecium</i> (40, including 38 VanA) <i>E. faecalis</i> (2)	Multivariate	Hematological malignancy, use of vancomycin, severity of illness ^c
252	Hepatology (included transplant recipients)	Various (colonization + infection) (38)	NA ^d	Multivariate	Length of hospital stay
161	Liver transplantation	Blood (54)	VanA (54)	Multivariate	Length of hospital stay

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^b Seven hospitals including both primary and tertiary care centers.

^c Based on APACHE II score.

^d NA, not available.

VRE developed urinary retention and urinary tract infection with a VRE strain that was found to be indistinguishable from the woman's isolate by PFGE (231). Thus, as colonized patients leave the hospital environment, the possibility that transmission might occur in the community cannot be discounted.

The situation in Europe is quite different from that in the United States. In Europe, VRE have been isolated from sewage and various animal sources (19, 139). It has been suggested that the use of glycopeptide-containing animal feeds in some regions of Europe may have contributed to such differences (184). In one study, VanA-resistant *E. faecium* was isolated from frozen poultry and pork and from the feces of 12 of 100 nonhospitalized inhabitants in a rural area (139). VanA VRE have also been found in the feces or intestines of other farm animals or pets, including horses, dogs, chickens, and pigs (65). These observations suggest a potential for VRE or the resistance genes of VRE to reach humans through the food chain or through contact with domesticated animals.

Colonization of healthy individuals with VRE does not necessarily indicate a risk of infection with these organisms. In a point-prevalence culture survey at one Belgian hospital in 1993, 3.5% of patient stool isolates were positive for VRE; however, to that point, no infections due to VRE had been encountered at that institution (111). Van der Auwera et al. reported that stool cultures from 11 (28%) of 40 healthy volunteers who were not health care workers and who had not taken antibiotics in the preceding year yielded a heterogeneous collection of isolates of vancomycin-resistant *E. faecium* (249). The same group also detected VRE in the stools of up to 64% of volunteers who had received oral glycopeptides in previous studies (249).

Risk Factors

Early studies dealing with the emergence of VRE in the United States revealed that most patients with VRE were in ICUs (51). However, VRE are now being seen with increasing frequency among patients with chronic renal failure or cancer,

organ transplant recipients, and patients who experience prolonged hospitalization (30, 43, 71, 114, 135, 137, 164, 247, 248). Several studies have used case-control methods and multivariate analysis to examine the risk factors for VRE infection among hospitalized individuals (Table 3). Among the risk factors that have emerged are longer duration of hospitalization (181, 235, 247), longer lengths of stay in ICU (176, 201), the need for intrahospital transfer to another ward (247), the need for surgical reexploration following liver transplantation (201), and the use of enteral tube feedings or sucralfate (235). While gastrointestinal tract colonization may precede infection in many patients, in one study stool surveillance culture positivity antedated infection in only half of the cases (255). This may in part reflect the limitations of surveillance cultures in detecting low densities of microorganisms. Occasionally, VRE will be detected in surveillance cultures of nose, throat, or mouth specimens in the absence of detectable rectal or perineal colonization. Other risk factors that have been associated with colonization or infection include previous antimicrobial therapy, exposure to contaminated medical equipment such as electronic thermometers, proximity to a previously known VRE patient, and exposure to a nurse who was assigned on the same shift to another known patient (30, 137, 164). Risk factors specifically associated with VRE infections such as bacteremia include malignancy, increased Acute Physiology and Chronic Health Evaluation (APACHE) II score, neutropenia, prolonged hospital stay, antibiotic therapy and preceding therapy with agents active against anaerobes, mean number of days on antibiotic therapy, renal insufficiency, and hospitalization on a hematologic malignancy/bone marrow transplantation service (30, 71, 121, 152, 179, 230). Parenteral vancomycin use and receipt of third-generation cephalosporins have been cited by others as risk factors for colonization or infection with VRE (61, 180, 181, 247). In a recent prospective cohort study using logistic regression, VRE colonization at the time of ICU admission was found to be associated with second- and third-generation cephalosporins, length of stay prior to surgical ICU

admission, more than one prior ICU stay, and history of solid-organ transplantation (198).

Oral vancomycin use may also be a risk factor for VRE colonization (29, 147, 165), and this has led to recommendations discouraging the use of this agent for the primary treatment of antibiotic-associated diarrhea (44). However, there is also recent evidence that metronidazole may not be a microbiologically innocuous alternative to oral vancomycin for the treatment of antibiotic-associated diarrhea. The use of oral or parenteral metronidazole (or other agents with significant antianaerobic activity) was noted as a risk factor for VRE bacteremia in one study (71), while others have suggested that metronidazole or clindamycin exposure is a risk factor for VRE acquisition (176).

Vancomycin most probably predisposes patients to colonization and infection with VRE by inhibiting the growth of the normal gram-positive bowel flora and by providing a selective advantage for VRE that may be present in small numbers in the individual's bowel (249; Murray, Editorial response). For example, Van der Auwera et al. found that administration of oral vancomycin or teicoplanin to individuals whose baseline stool specimens contained few or no detectable VRE led to recovery of VRE in large numbers, sometimes as much as 10^6 to 10^8 CFU/g of stool (249). The selective pressure exerted by the increasing use of vancomycin in the United States during the last 10 to 15 years has been extraordinary. For example, the amount of vancomycin used at one university hospital increased 20-fold from 1981 to 1991 (83).

Beezhold et al. reported a high prevalence of skin colonization with VRE (86%) among hospitalized patients with VRE bacteremia (21). Skin colonization with VRE was found to be associated with diarrhea (prior or present) or fecal incontinence in two studies (21, 263). The high prevalence of skin colonization might explain the importance of VRE as a cause of catheter-related sepsis. It may also increase the risk of cross-infection or blood culture contamination, which may also explain the frequent spontaneous resolution of bacteremia due to VRE (21).

Colonization and Infection

The emergence of vancomycin resistance in enterococci in addition to the increasing incidence of high-level enterococcal resistance to penicillin and aminoglycosides presents a serious challenge for physicians treating patients with infections due to these microorganisms (43, 121). Treatment options are often limited to combinations of antimicrobials or experimental compounds with unproven efficacy (125, 174). Resistance to cephalosporins and clindamycin occurs almost without exception. Few isolates are susceptible to currently available macrolides, and resistance to fluoroquinolones is now common (78). Once VRE are established in the hospital environment, their frequent resistance to multiple antibiotics makes it difficult to avoid further selective pressure in their favor (156).

In many affected institutions, most patients from whom VRE are recovered are colonized rather than infected with the organism (30, 135, 156, 176). The ratio of colonized to infected patients may reach as high as 10:1 at hospitals in which perirectal or rectal swab specimens from high-risk patients are screened for VRE (135, 176). In one study, 40% of organisms colonizing the gastrointestinal tract were *E. gallinarum*, but no infections were caused by this species (156). Infections caused by VRE often involve intra-abdominal sites, the urinary tract, the bloodstream, surgical sites, and vascular catheter sites.

VRE infections tend to occur in more debilitated or seriously ill hospitalized patients. Mortality rates in patients with

VRE bacteremia may reach 60 to 70% (70, 71, 247). Approximately half of these deaths may be attributable directly to the infection. Papanicolaou et al. found VRE infection to be a strong predictor of mortality in liver transplant patients (201). Linden et al. reported that enterococcal infection-related mortality was 46% in liver transplant recipients with VRE bacteremia, which was significantly greater than the 25% mortality observed in patients with vancomycin-susceptible enterococcal bacteremia (161). Patients with neutropenia, chronic renal failure, or other serious conditions and liver transplant recipients seem to be the most likely to experience prolonged bacteremia or to die as a result of VRE (179).

In other studies, comparison of patients with VRE and vancomycin-susceptible enterococcal bacteremias revealed no significant differences in mortality, especially after controlling for factors such as age and APACHE II score (30, 247, 255). There is no evidence that VRE are more virulent than vancomycin-susceptible strains of the same enterococcal species.

Although many nosocomial enterococcal bacteremias are polymicrobial, 80 to 90% of VRE bacteremias have been monomicrobial in some series (70, 161). These studies have revealed that some enterococcal bacteremias are associated with refractory hypotension (161) or hypotension with clinically significant hypoperfusion and organ dysfunction (70). Hypotension has also accompanied *E. faecalis* bacteremic superinfection observed during the treatment of *E. faecium* infection with dalfopristin-quinupristin (49).

In addition to bloodstream infections, which are often catheter related, VRE may be isolated from urine, intra-abdominal abscesses or surgical site infections, and various tubes and drains relating to infections within the abdomen. VRE are occasionally isolated from pleural fluid and rarely isolated from cerebrospinal fluid; generally this occurs following surgery or other instrumentation (80).

It is not always easy to assess the clinical significance of VRE in routine cultures or to differentiate colonization from infection. This is especially true for urine or when VRE are part of a polymicrobial infection. In some cases, attempts at treatment are not indicated. The extent to which VRE causes morbidity and mortality is often difficult to determine, because most affected patients have serious underlying diseases that cause substantial morbidity and death and because VRE are often recovered in mixed cultures with other potential pathogens (32).

Reservoirs

Although much has been learned about the epidemiology of VRE in recent years, a consensus regarding the most important reservoirs of VRE has not been reached. In 1993, Bates et al. noted that several hospitalized VRE patients in the United Kingdom had had little or no previous contact with medical institutions (J. Bates, Z. Jordens, and J. B. Selkon, Letter, *Lancet* 342:490–491, 1993). Subsequent investigations revealed that several of these patients resided on farms and that chickens and swine present on the farms were colonized with vancomycin-resistant *E. faecium*. VRE has subsequently been recovered from various animal sources in different European countries (processed chickens, chicken carcasses, pork meat) (140; Bates et al., Letter). The occurrence of VRE in such animals could be related to the fact that avoparcin (a glycopeptide) has been available as a feed additive for more than 15 years in the United Kingdom and other European countries (1, 260). It has been fed to broiler chickens, swine, and cattle (270). These findings suggest that contaminated food products may serve as a reservoir from which nonhospitalized individuals can acquire VRE.

In the United States, avoparcin is not a licensed feed additive for animals, and culture surveys of a limited number of chickens in several cities have failed to detect VRE (T. S. Harrison, S. Qaiyumi, J. G. Morris, Jr., and R. S. Schwalbe, Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J78, 1995; Murray, Editorial response). Further studies of animal-based food products are needed to determine if food items represent a community reservoir for VRE in this country.

At present, hospitalized patients with gastrointestinal carriage of VRE appear to be the major reservoir of the organism in the United States. Because most colonized patients are asymptomatic, this reservoir can easily go unnoticed unless surveillance culture specimens are obtained from patients at risk (32). The gastrointestinal tract is undoubtedly the major reservoir for *E. faecium*, but positive clinical specimens in the absence of fecal carriage provide evidence for direct exogenous acquisition rather than gastrointestinal colonization and subsequent endogenous infection (252). In one study, 33% of very ill liver patients who acquired vancomycin-resistant *E. faecium* had positive throat swabs (252). Colonization at this site may follow contamination by staff hands during mouth, tracheostomy, or endotracheal tube care. Oropharyngeal colonization may provide a source for cross-colonization, particularly if staff consider such manipulations to be "clean" and do not subsequently wash their hands. In another study, however, vancomycin-resistant *E. faecium* was not isolated from throat swabs of any patients known to be infected or colonized at other sites (121).

Environmental surfaces and medical equipment items in the patient's room frequently become contaminated with VRE and may also serve as a reservoir for the organism in the hospital. Examples of items that may be contaminated include patient gowns and linen, beds, bedside rails, overbed tables, floors, doorknobs, washbasins, glucose meters, blood pressure cuffs, electronic thermometers, electrocardiogram monitors, electrocardiograph wires, intravenous fluid pumps, and commodes (29, 30, 112, 137, 164, 167, 181, 235). Widespread environmental contamination by VRE is especially likely to occur in the rooms of patients who have diarrhea (30). In some studies, isolates from contaminated surfaces and from affected patients in the room have been shown to represent the same strains of VRE (29, 235). VRE may remain viable on such surfaces for days or weeks because the organisms are resistant to desiccation and extreme temperatures (30, 101, 138, 195). For example, VRE may survive for 5 to 7 days on countertops and have been recovered 24 h or more after experimental contamination of bedrails, telephone handpieces, or stethoscope diaphragms (195). Vancomycin-resistant *E. faecium* has also been isolated from a tourniquet 4 days after discharge of a colonized patient and from intravascular monitoring equipment after several days in storage (30, 161, 176). VRE have been recovered from the hands of personnel by some investigators (250) but not by others (30, 181, 252). In a few instances, health care workers have been found to have gastrointestinal colonization with VRE, but the epidemiologic significance of this finding is unclear (121, 135).

Montecalvo et al. emphasized that patients may remain colonized with VRE for weeks or months and are often still colonized at the time of readmission to the hospital (176). Their findings support earlier reports of persistent VRE colonization among high-risk patients (30, 114, 164). Green et al. reported that nearly 60% of liver transplant patients with VRE remained colonized for 12 weeks or more (115), and Livornese et al. found that a majority of patients remained colonized for more than 3 months (164). Occasional patients who remained

VRE culture positive for as long as 1 year have been reported (30). According to the study of Montecalvo et al., some patients were persistently colonized with the same VRE strain, as demonstrated by PFGE, whereas others were positive for more than one strain during the follow-up period (176). Because persistently colonized patients may reintroduce VRE into a facility on multiple occasions, hospitals should develop means of prompt identification of such patients at the time of readmission so that they can be placed in isolation pending repeat surveillance cultures (30, 44).

Modes of Transmission

Transmission of VRE by health care workers whose hands become transiently contaminated with the organism while caring for affected patients is probably the most common mode of nosocomial transmission. This concept is supported by the recovery of VRE and other resistant enterococci from cultures of specimens from the hands of health care workers (247, 267).

Transmission of VRE may also occur by way of contaminated medical equipment, although this is probably much less important than transmission by the hands of personnel. Electronic thermometers contaminated with the outbreak strain were epidemiologically implicated in an outbreak described by Livornese et al. (164). The spread of VRE via bedpan washer machines has also been reported (P. R. Chadwick and B. A. Openheim, Letter, Lancet 344:685, 1994). Nosocomial transmission of VRE has been attributed to the use of fluidized microsphere beds from which multiply resistant strains were recovered despite repeated attempts at decontamination by the manufacturers (100). Enterococci have been recovered from 7 to 30% of environmental cultures during several outbreaks (30, 71, 137, 181, 263, 267). Since enterococci may remain viable for several days to weeks on dry surfaces, it seems plausible that contaminated surfaces may act as a source from which personnel may contaminate their hands or clothing (30). However, further studies are necessary to determine the extent to which these items contribute to the transmission of VRE (32).

Disposable cover gowns worn by personnel who care for VRE patients have also been shown to be contaminated with the patient's organism (29). Presumably, the clothing of the personnel who do not wear cover gowns may also become contaminated with VRE. However, at present there is no conclusive proof that VRE are spread by contaminated clothing. There is no proof that enterococci, including VRE, are spread by the airborne route. Recovery of VRE from animal sources in parts of Europe suggests that food-borne transmission may occur in certain geographic areas (260; Bates et al., Letter). However, conclusive proof of food-borne transmission of VRE in Europe (or other areas) is not yet available.

Prevention and Control Measures

The epidemiology of VRE has not been elucidated completely; however, as mentioned above, certain patient populations are at increased risk for VRE infection or colonization. These include critically ill patients or those with severe underlying disease or immunosuppression, such as ICU patients or patients in oncology or transplantation wards, those who have had an intra-abdominal or cardiothoracic surgical procedure, those with an indwelling urinary or central venous catheter, and those who have had a prolonged hospital stay or received multiple antimicrobial agents (30, 33, 43, 104, 121, 137, 177). Because enterococci are part of the normal flora of the gastrointestinal tract and the female genital tract, most infections with these organisms have been attributed to the patient's

TABLE 4. Measures for reducing nosocomial transmission of VRE^a

Control measures	Hospitals without VRE	Hospitals with low prevalence of VRE	Hospitals with high prevalence of VRE
Reduce excessive use of antibiotics	Strongly recommended	Strongly recommended	Strongly recommended
Educational programs for personnel	Strongly recommended	Strongly recommended	Strongly recommended
Surveillance			
Test all enterococcal isolates for resistance to vancomycin	Periodically	Routinely	Routinely
Point prevalence culture surveys on affected wards	Not applicable	Culture roommates or, preferably, all patients on ward when new case occurs	Perform periodic surveys in high-risk units; consider screening patients on admission to high-risk units
Identify patients with VRE at the time of readmission	Not applicable	When possible, use computerized demographic file to detect VRE patients on admission	Desirable, but may not be practical in all facilities with high prevalence of VRE
Placement of patients with VRE	Not applicable	Place colonized or infected patients in a private room	Isolate or cohort VRE patients in high-risk unit
Gloves	Standard precautions ^b	Wear gloves when entering rooms of VRE patients	Wear gloves when entering rooms of VRE patients and consider wearing gloves to care for all patients in high-risk units
Gowns	Standard precautions ^b	Wear gown if substantial contact with patient or environment is anticipated	Routine wearing of gowns, in addition to gloves, may have little added benefit
Hand washing and hand hygiene	Standard precautions ^b	After removing gloves, wash hands with antiseptic soap or use waterless antiseptic agent	After removing gloves, wash hands with antiseptic soap or use waterless antiseptic agent
Noncritical patient care equipment items (e.g., stethoscopes, sphygmomanometers)	Standard precautions ^b	Dedicate noncritical items to a single patient or cohort of patients	Dedicate noncritical items to a single patient or cohort of patients
Discontinuing barrier precautions	Not applicable	Require 3 consecutive negative cultures for VRE from stool or rectal swab specimens and from other body sites colonized with VRE taken 1 wk or more apart	Require 3 consecutive negative cultures for VRE from stool or rectal swab specimens and from other body sites colonized with VRE taken 1 wk or more apart
Housekeeping	Use standard cleaning and disinfection procedures	Emphasize the importance of cleaning and disinfection procedures	Consider obtaining cultures before and after cleaning to ensure the efficacy of procedures used

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^b Standard precautions, as defined by the CDC HICPAC recommendations (107).

endogenous flora (185). However, recent reports have demonstrated that enterococci, including VRE, can be spread by direct patient-to-patient contact or indirectly via transient carriage on the hands of personnel (30), contaminated environmental surfaces (30, 137), or patient care equipment (164).

In addition to the existing problem with VRE, the potential emergence of vancomycin resistance in clinical isolates of *S. aureus* or *S. epidermidis* is a serious public health concern. The *vanA* gene, which is frequently plasmid borne, can be transferred in vitro from enterococci to a variety of gram-positive microorganisms including *S. aureus* (193).

In response to the dramatic increase in vancomycin resistance in enterococci, the Subcommittee on Prevention and Control of Antimicrobial-Resistant Microorganisms in Hospitals of the CDC Hospital Infection Control Practices Advisory Committee (HICPAC) had several meetings in 1993 and 1994. In an effort to control the nosocomial transmission of VRE, HICPAC published recommendations in February 1995 (44). These recommendations mainly focused on (i) prudent use of vancomycin, (ii) education of hospital staff, (iii) effective use of the microbiology laboratory, and (iv) implementation of infection control measures (including the use of gloves and gowns and isolation or cohorting of patients, as appropriate to specific conditions).

To minimize nosocomial transmission of VRE, hospitals must use a multidisciplinary approach that requires participation by a variety of departments and personnel (Table 4) (32, 44).

Prudent use of vancomycin. Efforts to control antibiotic-resistant organisms generally focus on decreasing the use of antibiotics and decreasing the opportunities for the spread of organisms between individuals. The logic behind efforts to decrease antibiotic use is that the presence of an antibiotic provides a tremendous advantage to a resistant organism and can increase the number of resistant bacteria manyfold (186). The greater the number of resistant bacteria in a given clinical sample, the easier it is for that resistant organism to be transmitted to another person. This makes efforts at decreasing transmission more important but also more difficult.

Vancomycin use has been reported consistently as a risk factor for colonization and infection with VRE (30, 121, 164, 177) and may increase the possibility of the emergence of vancomycin-resistant *S. aureus* or *S. epidermidis*. Encouraging the appropriate use of oral and parenteral vancomycin is an important component of HICPAC recommendations. In an effort to bring about more prudent use of antibiotics, HICPAC emphasizes the importance of education of medical staff and students about the situations in which the use of vancomycin is

considered appropriate. It also gives a long list of situations in which vancomycin use should be discouraged. According to HICPAC recommendations, situations in which the use of vancomycin is appropriate or acceptable are as follows (44): (i) for treatment of serious infections due to β -lactam-resistant gram-positive microorganisms; (ii) for treatment of infections due to gram-positive microorganisms in patients with serious allergy to β -lactam antimicrobials; (iii) when antibiotic-associated colitis fails to respond to metronidazole therapy or is severe and potentially life-threatening; (iv) prophylaxis, as recommended by the American Heart Association, for endocarditis following certain procedures in patients at high risk for endocarditis; and (v) prophylaxis of major surgical procedures involving the implantation of prosthetic materials or devices, e.g., cardiac and vascular procedures and total hip placement, at institutions with a high rate of infections due to methicillin-resistant *S. aureus* (MRSA) or methicillin-resistant *S. epidermidis* (MRSE). A single dose administered immediately before surgery is sufficient unless the procedure lasts more than 6 h, in which case the dose should be repeated. Prophylaxis should be discontinued after a maximum of two doses.

Situations in which the use of vancomycin should be discouraged include the following: (i) routine surgical prophylaxis other than in a patient with life-threatening allergy to β -lactam antibiotics; (ii) empirical antimicrobial therapy for a febrile neutropenic patient, unless there is strong evidence at the outset that the patient has an infection due to gram-positive microorganisms (e.g., inflamed exit site of a Hickman catheter) and the prevalence of infections due to MRSA in the hospital is substantial; (iii) treatment in response to a single blood culture positive for coagulase-negative staphylococci, if other blood cultures drawn in the same time frame are negative, i.e., if contamination of the blood culture is likely (because contamination of blood cultures with members of the skin flora, e.g., *S. epidermidis*, may cause vancomycin to be administered to patients inappropriately, phlebotomists and other personnel who obtain blood cultures should be trained properly to minimize microbial contamination of specimens); (iv) continued empirical use for presumed infections in patients whose cultures are negative for β -lactam-resistant gram-positive microorganisms; (v) systemic oral or local (e.g., antibiotic lock) prophylaxis of infection or colonization of indwelling central or peripheral vascular catheters; (vi) selective decontamination of the digestive tract; (vii) eradication of MRSA colonization; (viii) primary treatment of antibiotic-associated colitis; (ix) routine prophylaxis in very low-birth-weight infants; (x) routine prophylaxis in patients on continuous ambulatory peritoneal dialysis or hemodialysis; (xi) treatment (chosen for dosing convenience) of infections due to β -lactam-susceptible gram-positive microorganisms in patients with renal failure; and (xii) use of vancomycin solution for topical application or irrigation.

Vancomycin use in hospitals increased significantly over the 1980s (165). As noted above, there is some evidence that restricting the use of this agent may assist in the control of VRE outbreaks. Further study is required to determine the most effective methods for influencing the prescribing practices of physicians, although a variety of techniques may be useful (85, 237, 238). In addition, key parameters of vancomycin use can be monitored through the hospital's quality assurance and improvement process or as a part of the drug utilization review of the pharmacy and therapeutics committee and the medical staff.

A factor that may complicate our ability to reduce or eliminate VRE by decreasing vancomycin use is that VRE are often resistant to multiple other antimicrobial agents as well. Administration of any of these agents could potentially provide

a selective advantage for VRE and enhance their survival (186). The use of third-generation cephalosporins has long been recognized as a risk factor for enterococcal infections in general. Several studies have shown that receipt of third-generation cephalosporins and use of agents with significant anti-aerobic activity are risk factors for colonization or infection with VRE (71, 176, 180, 181, 247). Other measures that have been suggested for the control of VRE outbreaks include formulary policies discouraging the use of third-generation cephalosporins and agents most likely to cause *C. difficile* colitis (209).

Education programs. Continuing educational programs for hospital staff (including attending and consulting physicians, medical residents, students, pharmacy personnel, nurses, laboratory personnel, and other direct patient caregivers) should include information about the epidemiology of VRE and the potential impact of this pathogen on the cost and outcome of patient care (44). Because detection and containment of VRE require a very aggressive approach and high performance standards for hospital personnel, special awareness and educational sessions may be indicated.

Role of the microbiology laboratory in the detection, reporting, and control of VRE. Early detection of patients colonized or infected with VRE is an essential component of any hospital program designed to prevent nosocomial transmission of VRE (32). Once the prevalence of VRE reaches high levels within an institution, prevention of transmission is more difficult. The microbiology laboratory is the first line of defense against the spread of VRE in the hospital. The ability of the laboratory to identify enterococci and to detect vancomycin resistance promptly and accurately is essential in recognizing VRE colonization and infection and avoiding complex, costly containment efforts that are required when recognition of the problem is delayed (44). In addition, cooperation and communication between the laboratory and the infection control program will facilitate control efforts substantially. In many hospitals, the first cases of VRE have been detected by isolating VRE from clinical specimens submitted to the laboratory for clinical purposes (104, 137). Although some hospital laboratories continue to perform antimicrobial susceptibility testing only on enterococci recovered from normally sterile body sites such as blood or urine, this practice is no longer appropriate for areas in which VRE have been encountered (44; Murray, Editorial response). Only 45 to 50% of VRE isolated from clinical specimens during VRE outbreaks have been recovered from blood or urine specimens. Accordingly, once VRE have been detected in a hospital, enterococci recovered from all body sites should be tested for susceptibility to vancomycin (44; Murray, Editorial response). Hospitals that have not yet detected VRE but are located in (or receive patients from) geographic areas where VRE have been encountered should strongly consider performing susceptibility tests on all enterococcal isolates (Table 4) (44).

Susceptibility tests that detect vancomycin resistance accurately must be used, or the prevalence of VRE may be underestimated (188, 241, 242, 244). Laboratories using disk diffusion should incubate plates for 24 h and read zones of inhibition under transmitted light (188, 242). MICs can be determined by agar dilution, agar gradient dilution, broth macrodilution, or manual broth microdilution (188, 242). These systems should also be incubated for 24 h. Some of the fully automated methods of testing enterococci for resistance to vancomycin are unreliable, especially in detecting VRE isolates containing the VanB resistant determinant. In a recent study evaluating the accuracy of eight currently available susceptibility test methods (agar dilution, disk diffusion, E-test,

agar screen plate, Vitek GPS-TA and GPS-101, and MicroScan overnight and rapid panels), it was shown that *vanA* VRE were detected by all methods but *vanB* VRE were often not detected by Vitek GPS-TA and MicroScan rapid (sensitivities, 47 and 53% respectively) (84). All methods except the E-test and the agar screen continue to show problems in the detection of VanC1 and VanC2 VRE. The agar screen appears to be the most reliable and easy method for routine screening, if detection of VanA-, VanB-, VanC1-, and VanC2-mediated resistance in enterococci is required. The new Vitek GPS-101 shows improved sensitivity compared to the Vitek GPS-TA without significant loss of specificity (84). When VRE are isolated from a clinical specimen, vancomycin resistance should be confirmed by repeating antimicrobial susceptibility testing by any of these recommended methods, especially if VRE isolates are unusual in the hospital. While performing confirmatory susceptibility tests, the patient's primary caregiver, patient care personnel on the ward on which the patient is hospitalized, and infection control personnel must be immediately notified about the presumptive identification of VRE, so that the patient can be placed on appropriate isolation precautions promptly (44). This preliminary report must be followed by the (final) result of the confirmatory test.

Enterococci may also be tested for vancomycin resistance by using PCR assays designed to detect the genes responsible for glycopeptide resistance in these organisms (51, 242). Such tests may be particularly helpful in detecting VanB- or VanC-containing strains with low-level resistance to vancomycin (242). Testing VRE isolates for susceptibility to teicoplanin by using simple disk diffusion tests will differentiate between VanA (teicoplanin-resistant) and VanB (teicoplanin-susceptible) strains in most instances. However, occasional teicoplanin-resistant VanB-type strains have been reported. Surveillance cultures for VRE are time-consuming and expensive for the laboratory to perform. Recently, it has been reported that PCR may be a cost-effective alternative to surveillance cultures for VRE in some hospitals (226). However, in using the alternative, the laboratory may lose the ability to perform strain-typing studies with the organisms in their institutions (226).

It is important to establish whether the emergence of VRE in a hospital has been caused by the spread of a single strain or by the simultaneous appearance of several different clones, because this can affect the control measures that may be given priority (32). Since most strains of VRE are resistant to multiple drugs, antimicrobial susceptibility patterns lack discriminatory power. A variety of molecular typing methods have been used to establish the degree of clonal relatedness of VRE, including ribotyping, plasmid analysis, PFGE, arbitrarily primed PCR, and examination of hybridization patterns obtained with probes, such as insertion sequences (IS6770) or *vanA* and *vanB* gene probes (16, 19, 30, 51, 69, 104, 164, 167). A combination of genotypic methods such as PFGE plus plasmid analysis is likely to yield the most accurate information about the number of strains present in an institution and the patterns of transmission (51, 167).

Implementation of infection control measures. The current isolation precautions recommended by HICPAC to prevent patient-to-patient transmission of VRE are as follows.

(i) Place VRE-infected or -colonized patients in single rooms or in the same room as other patients with VRE (30).

(ii) Wear clean nonsterile gloves when entering the room of a VRE-infected or -colonized patient (30, 137, 164). During the course of caring for a patient, a change of gloves may be necessary after contact with material that may contain high concentrations of VRE (e.g., stool).

(iii) Wear a clean nonsterile gown when entering the room of a VRE-infected or -colonized patient if substantial contact with the patient or environmental surfaces in the patient's room is anticipated or if the patient is incontinent or has diarrhea, an ileostomy, a colostomy, or wound drainage not contained by a dressing (30).

(iv) Remove gloves and gowns before leaving the patient's room, and wash hands immediately with an antiseptic soap or use a waterless antiseptic agent (66, 133, 192). Hands can be contaminated via glove leaks or during glove removal, and bland soap is relatively ineffective in removing VRE from the hands (40, 63, 141, 143, 205).

(v) Ensure that after glove and gown removal and hand-washing, clothing and hands do not contact environmental surfaces potentially contaminated with VRE (e.g., door knob or curtain) in the patient's room (30, 137).

Although it is not recommended by HICPAC, some hospitals require that gowns and gloves be worn routinely by all personnel entering a VRE patient's room (30, 121). Anecdotal experience in an ICU in which outbreaks caused by a single clone of VRE occurred revealed that routinely wearing gowns and gloves before entering the rooms of patients, combined with other infection control measures, was effective in terminating the outbreaks (29, 30). Handwerger et al. also reported that the use of gowns in addition to the other infection control procedures terminated the outbreak (121). A recent prospective study conducted in an ICU in which multiple strains of VRE were highly endemic, however, found that routine use of gloves and gowns was no more effective in interrupting transmission than was universal use of gloves alone (235). The fact that routine use of gowns did not seem to provide additional protection against VRE transmission in the latter study may have been caused partially by the fact that heavy environmental contamination did not occur during the study period. Further studies are needed to establish the circumstances in which routine use of gowns by personnel provides additional protection against the spread of VRE.

In addition to these isolation precautions, the use of non-critical items such as stethoscopes, sphygmomanometers, or rectal thermometers should be dedicated to a single patient or cohort of patients infected or colonized with VRE (164). If such devices are to be used on other patients, they should first be adequately cleaned and disinfected (251). Culture of stools or rectal swabs of roommates of patients newly found to be infected or colonized with VRE must be performed to determine the colonization status and to see whether isolation precautions are necessary. Additional screening of patients on the ward can be performed at the discretion of the infection control staff. A policy deciding when patients infected and/or colonized with VRE can be removed from isolation precautions should be adopted. The optimal requirements remain unknown. However, since VRE colonization may persist indefinitely, stringent criteria may be appropriate, e.g., VRE-negative cultures on at least three consecutive occasions, 1 week or more apart, for all cultures from multiple body sites (including stool, rectal swab, perineal area, axilla or umbilicus, and wound, Foley catheter, and/or colostomy sites if present) (121). A system of highlighting the records of infected or colonized patients should be established so that they can be recognized and placed on isolation precautions promptly upon readmission to the hospital, because patients with VRE may remain colonized for long periods following discharge from the hospital. Ideally, this information should be computerized so that placement of colonized patients will not be delayed due to unavailability of the patients' medical records. Local and state health departments should be consulted in developing a plan

for the discharge of VRE-infected or colonized patients to nursing homes, other hospitals, or home health care as part of a larger strategy for handling patients with resolving infections and patients colonized with antimicrobial-resistant microorganisms.

The HICPAC has some additional recommendations for hospitals with endemic VRE or continued VRE transmission despite the implementation of measures (44). These are as follows.

(i) Control efforts should initially be focused in ICUs and on areas where the VRE transmission rate is highest (121). Such units may serve as a reservoir for VRE, from which VRE spreads to other wards when patients are well enough to be transferred.

(ii) Where feasible, staff who provide regular care to patients should be cohorted to minimize the movement of health care givers between VRE-positive and VRE-negative patients.

(iii) In conjunction with careful epidemiologic studies and upon the direction of the infection control staff, personnel should be examined for chronic skin and nail problems. Hand and rectal swab cultures should be performed on specimens obtained from them. VRE-positive personnel epidemiologically linked to VRE transmission should be removed from the care of VRE-negative patients until their carrier state has been eradicated.

(iv) The results of several enterococcal outbreak investigations suggest a potential role for the environment in the transmission of enterococci (30, 164, 267). Institutions experiencing ongoing VRE transmission should verify that the hospital has adequate procedures for the routine care, cleaning, and disinfection of environmental surfaces (e.g., bedrails, charts, carts, doorknobs, faucet handles, and bedside commodes) and that these procedures are being followed by housekeeping personnel. Some hospitals may elect to perform focused environmental cultures before and after cleaning rooms housing patients with VRE to verify the efficacy of hospital policies and procedures. All environmental culturing should be approved and supervised by the infection control program in collaboration with the clinical laboratory (30, 164, 267).

(v) Representative VRE isolates should be sent to reference laboratories for strain typing by PFGE or other suitable techniques to aid in defining reservoirs and patterns of transmission.

Some hospitals have been slow to implement the new HICPAC recommendations for isolation of patients with VRE and continue to use more traditional isolation systems such as "contact isolation" (initially described in 1983) or "body substance isolation" (106). Unfortunately, a number VRE outbreaks have continued despite the use of these policies (30, 137, 177, 181, 224, 247). Therefore, hospitals that have not already implemented barrier precautions recommended by HICPAC should strongly consider doing so.

Strict implementation of infection control measures has contributed to the control of VRE outbreaks (29, 209). Other authors have been less impressed with the value of contact precautions in reducing the rates of VRE acquisition in institutions where VRE colonization is endemic (145, 176, 181, 255). Even skeptics agree, however, that rates might have been increased further in the absence of infection control efforts. Unfortunately, breaches in infection control protocols occur commonly. In one study, breaks in protocol were noted in 72% of monitored patient interactions (30). A number of other studies have demonstrated that compliance of health care workers with recommended barrier precautions is often sub-

optimal (89, 151, 181, 235). Accordingly, hospitals that experience difficulties in controlling the nosocomial transmission of VRE should consider developing systems for monitoring and improving the compliance of personnel with recommended barrier precautions.

Control of VRE in long-term-care facilities. There are a few studies indicating that colonized residents of LTCFs may serve as a reservoir for acute-care hospitals (27, 35; Elizaga et al., Abstract; Quale et al., Abstract; Revuelta et al., Abstract; Sargent et al., Abstract). These studies suggest that VRE may not be a frequent cause of infection in residents of LTCFs (58). The cost of control measures that are recommended for acute care may be prohibitive in LTCFs, and these measures are impractical for use in LTCFs. VRE have not yet been reported to be a cause of serious illness in LTCF patients. It is known that highly compromised patients are potential candidates for serious VRE infection. This is not likely to be true of the vast majority of LTCF residents. Based on these observations, The Society for Healthcare Epidemiology of America Committee on Long-Term Care decided to develop a position document about VRE in LTCFs. This document was recently published (58).

The Committee believes that patients who are infected or colonized with VRE may be cared for safely in an LTCF with minimal risk of development of nosocomial infection in other patients and that the task of carrier identification and proper isolation probably is borne most appropriately by acute-care institutions (58). According to the recommendations of the Committee, the general approach to the control of VRE in LTCFs is summarized as follows (58).

(i) Employee education about basic infection control and VRE is essential to any effort to control these organisms.

(ii) If an outbreak of infection appears to be under way, surveillance cultures of swabs of the rectum or perirectal area and wounds for VRE may be appropriate. Otherwise, they are unlikely to be cost-effective and are not recommended.

(iii) When a patient infected or colonized with VRE is transferred from an LTCF to an acute-care facility, this information should be provided to the receiving institution.

(iv) As an initial recommendation, the Committee advises continuing VRE isolation until at least two rectal cultures (or wound cultures) taken on separate days are found to be negative for the organism.

(v) If possible, patients infected or colonized with VRE should be placed in private rooms. If the patient must share a room, a roommate colonized with the same organism is preferred. If there is no private room available, a patient with VRE (who is continent of stool, does not have diarrhea, and does not have an open wound infected or colonized with VRE) can be placed in the same room with another patient. Although no data are available, it would be appropriate that the other patient not be severely immunocompromised (i.e., not have had organ transplantation, not be neutropenic, not be suffering from severe acute or chronic illness, and not have been treated recently with multiple or broad-spectrum antibiotics), not have open wounds, not be receiving antibiotics, and not have an indwelling catheter or other drainage device. Similar precautions would be appropriate if an infected or colonized patient must share a bathroom with another patient. Careful handwashing is necessary, and caring for the noncolonized roommate before contact with the VRE-colonized patient or his environment would be appropriate.

(vi) Gloves (clean and nonsterile) are required before contact with a colonized or infected patient, his or her secretions, and inanimate environment within the room. Gloves should be

changed after contact with materials that have a high concentration of microorganisms (e.g., stool) and before contact with the roommate or his or her immediate environment. Hands should be washed with an antiseptic agent containing chlorhexidine or alcohol after the gloves are removed.

(vii) Gowns are required if it is expected that the health care worker's clothing will have material contact with the patient, patient's secretions, or environmental surfaces. Gowns are especially important if a patient has diarrhea or a wound with drainage not contained in a dressing. Care must be taken to avoid environmental contact by clothing after the gown is removed. Gowns must be disposed of in a way that will minimize contamination of the environment.

(viii) Patient transport should be limited to situations required for medical care, and precautions must be continued, to prevent transmission to other patients and to prevent contamination of environmental surfaces. Room restrictions probably are appropriate only for patients with wound drainage not contained in a dressing or for those who are incontinent or who have diarrhea.

(ix) Patient care equipment should, if possible, be dedicated to a single patient. If this is impossible, appropriate cleaning and disinfection should be done between patients. The use of individual thermometers is strongly encouraged. Areas that the patient may contaminate (e.g., bedrails, bedside tables, commodes, soap dispensers, faucets, door handles, etc.) should be cleaned frequently (at least daily) with an appropriate disinfectant.

(x) Recommendations for prudent vancomycin use should be followed.

Surveillance cultures. Performing special surveillance cultures of patients to detect gastrointestinal colonization not identified by clinical cultures has often proved useful during outbreaks and should be considered an essential component of successful VRE control programs (29, 30, 44, 71, 137, 164, 181, 247). Failure to perform periodic prevalence surveys may result in poor control of VRE in hospitals. Prevalence surveys may be limited (culturing samples only from roommates of known VRE cases) or more comprehensive (culturing samples from all patients on a ward in which a newly discovered case has occurred or obtaining periodic samples for culture from high-risk patients such as those in ICUs, hematology-oncology wards, or transplantation units) (9, 29, 144, 189). During outbreaks, more comprehensive studies are desirable. Other strategies to identify colonized patients include screening stool specimens submitted for *C. difficile* toxin assays for VRE and screening rectal or perirectal swab specimens obtained from patients admitted from high-risk institutions (hospitals and LTCFs in which VRE are endemic). Perirectal cultures seem to have a sensitivity similar to rectal cultures for detecting colonized individuals (253).

Surveillance cultures of stool, rectal, or perirectal specimens should be inoculated onto selective media containing vancomycin (64, 121, 135, 181, 247, 253). Although brain heart infusion agar plates containing vancomycin are useful for confirming vancomycin resistance when testing isolated colonies of enterococci, they are not appropriate for screening stool specimens for VRE, because they support the growth of many other organisms.

Attempts to eradicate gastrointestinal colonization. There has been interest in eradication of gastrointestinal colonization with VRE for the following reasons (80): (i) to decrease the subsequent risk of infection in the individual patient, (ii) to minimize inconvenience to the patient and costs to the hospital associated with infection control procedures applicable to col-

onized patients, and (iii) to reduce the reservoir of VRE in the institutional environment.

Combinations of novobiocin with doxycycline or tetracycline failed to eradicate VRE from the stools of seven of eight treated patients (178). Two groups reported more promising results with oral bacitracin. In one study, treatment cleared VRE from stools in six of eight patients, with one relapse; in the other study, bacitracin cleared VRE in eight of eight patients, with two recurrences (47, 197). In another study, however, while combination therapy with bacitracin plus doxycycline initially cleared VRE from the stools of all treated patients, with longer follow-up only 33% remained free of detectable VRE, a proportion comparable to that in an untreated control group (M. R. Weinstein, J. Brunton, I. Campbell, et al., Program Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J10, 1996). Although some patients appear to have responded to these attempts at decolonization, no regimen has been uniformly effective in eradicating VRE from the gastrointestinal tract (32). Determination of whether VRE can be eradicated from the gastrointestinal tract by antimicrobial chemotherapy will require prolonged observation and use of enrichment techniques, or possibly even glycopeptide challenge, to detect low densities of VRE remaining in the stool before any regimen can be considered effective.

TREATMENT

Serious enterococcal infections (e.g., bacteremia and endocarditis) require treatment with a bactericidal combination of antibiotics that should include a penicillin (e.g., ampicillin or penicillin G) to which the *Enterococcus* isolate is susceptible and an aminoglycoside (e.g., gentamicin or streptomycin) to which the *Enterococcus* isolate does not exhibit high-level resistance (127). Vancomycin in combination with an aminoglycoside has demonstrated synergistic activity against enterococci both in vitro and in vivo (256), and it is recommended as the drug of choice in patients with serious penicillin allergy or in the treatment of ampicillin- and penicillin-resistant strains of bacteria (127). However, enterococci are becoming increasingly resistant to traditional antibiotic therapy. In addition to high-level aminoglycoside resistance and ampicillin resistance, rapid spread of vancomycin resistance has resulted in limited therapeutic alternatives (182, 185).

Treatment of infections due to VRE, especially *E. faecium*, is extremely problematic, because these organisms are resistant to multiple antibiotics. Penicillin or ampicillin with or without a synergizing aminoglycoside would be a reasonable choice in the nonallergic patient infected with vancomycin-resistant *E. faecalis*. Almost all *E. faecalis* strains are at least moderately susceptible to ampicillin. Therefore, if vancomycin resistance emerged predominantly in *E. faecalis*, the treatment of most of these infections could be relatively easy. Unfortunately, vancomycin resistance has preferentially appeared in *E. faecium*, which is inherently more resistant to penicillin and ampicillin. However, it should be remembered that many *E. faecium* strains are only moderately resistant to ampicillin. Routine testing by the clinical laboratory will classify an *E. faecium* strain as ampicillin resistant if it is able to grow in 16 µg of ampicillin per ml. Higher concentrations are not routinely tested. However, if the organism can be inhibited by 32 µg of ampicillin per ml, it may be worthwhile to use ampicillin, since it should be possible to exceed this concentration severalfold in vivo with high doses (186). Therefore, when faced with an infection by a strain of *E. faecium* that is resistant to both ampicillin and vancomycin, it would be appropriate to ask the laboratory to perform additional susceptibility tests to deter-

mine the MIC of ampicillin. While the level of resistance at which no benefit will be derived has not been established, based on the half-life and peak drug levels, it will likely be difficult to exceed a concentration of ≥ 128 $\mu\text{g/ml}$ of ampicillin in serum for a protracted period (186). Strains for which the ampicillin MICs are >100 $\mu\text{g/ml}$ are now common, and this concentration is close to the limit of concentrations achievable in the serum. Mekonen et al. described the failure of ampicillin at a total dose of 20 g/day (mean level in serum, 103 $\mu\text{g/ml}$) combined with gentamicin to clear VRE bacteremia in a liver transplant patient. Substitution of ampicillin-sulbactam at 30 g/day (equivalent to 20 g of ampicillin; mean ampicillin level in serum, 130 $\mu\text{g/ml}$) led to clearing of the bacteremia (168). The authors attributed this success to the slightly better activity of ampicillin combined with sulbactam compared with that of ampicillin alone against the clinical isolate of *E. faecium* (MIC, 32 and 64 $\mu\text{g/ml}$, respectively). In this case, β -lactamase production was not an issue. It is known that some strains are approximately one dilution more susceptible to ampicillin when tested in the combination (80). However, it seems doubtful whether this approach would have predictable utility in general. If the infecting VRE is highly resistant to ampicillin, there are few treatment options and one should ask the laboratory to test various other antimicrobials including tetracyclines, erythromycin, chloramphenicol, high levels of aminoglycosides, rifampin, fluoroquinolones, novobiocin, and, for urinary tract infection, nitrofurantoin (186). When enterococci have high-level resistance to both gentamicin and streptomycin, no regimen currently available is likely to produce a reliable bactericidal effect. Inconsistent results have been reported with high-dose ampicillin alone, vancomycin, teicoplanin, or ciprofloxacin, either alone or in combination with a penicillin or daptomycin (76, 127, 246). Currently, it is not known what constitutes the best therapy for enterococcal endocarditis due to strains with high-level resistance to all aminoglycosides. A reasonable approach would be continuous infusion of a high dose of ampicillin, probably for longer than the usual 6 weeks (73, 183). Valvular replacement may play a role in treating relapsing or intractable disease (93). Treatment options for multiple-drug-resistant enterococci are extremely limited, and agents to which they may appear susceptible are at best bacteriostatic, of unproven efficacy, or associated with toxicity (200). Thus, optimal therapy for these patients remains unknown.

Chloramphenicol is one of the few agents that retains *in vitro* activity against many strains of multiple-drug-resistant *E. faecium*. This agent has been used with limited (no reduction in mortality) or modest success in the treatment of VRE infections (200, 201). This was illustrated in a retrospective review of 16 patients with serious VRE infections (e.g., bacteremias and abscesses) who were treated with chloramphenicol at a university teaching institution (194). All the isolates were resistant to several antibiotics but were susceptible to chloramphenicol. Although 8 of 14 evaluable patients had some clinical response, 3 were classified as microbiological failures despite achieving drug levels in serum in the therapeutic range. One death was attributed to overwhelming sepsis due to VRE. The specific contribution of chloramphenicol to patient outcome could not be determined, since the response rate to therapy was confounded by numerous medical problems and multiple concomitant antimicrobials and interventions (e.g., drainage and debridement).

Teicoplanin is another glycopeptide that is active *in vitro* against most VanB-type enterococci. It has shown some efficacy in animal models of endocarditis when combined with an aminoglycoside to which the infecting strain is not highly re-

sistant. However, Hayden et al. described the *in vivo* development of teicoplanin resistance in a VanB *E. faecium* isolate (126). This finding has raised concern about this treatment option and has limited the therapeutic efficacy of this agent.

A number of new approaches to the treatment of VRE infections including β -lactam- β -lactam, β -lactam-glycopeptide, and β -lactam-fluoroquinolone combinations have been explored in experimental animal models (34, 42, 265). Each approach has limitations. The combination of a glycopeptide and a β -lactam is an interesting one whose use derives from the observation that some strains of *E. faecium*, although resistant to ampicillin and vancomycin, are still inhibited by the combination of the two. For such strains, the MIC of ampicillin decreases in the presence of vancomycin. This is possibly because the cell, in order to use the vancomycin-induced D-Ala-D-Lac-containing precursor, must shift to using a different cell wall synthesis enzyme (high-molecular-weight PBPs as described above). If this enzyme is more easily inhibited by ampicillin, the MIC of ampicillin would decrease (186). The laboratory can predict which strains will show this interaction by comparing the results of ampicillin disk susceptibility using agar plates with and without vancomycin or by determining the susceptibility to ampicillin using broth with and without vancomycin. Unfortunately, many ampicillin-resistant, vancomycin-resistant *E. faecium* strains do not show this phenomenon; even with those that do, subpopulations resistant to the combination usually exist and can be preferentially selected after exposure to the combination (186). The combination of ceftriaxone, vancomycin, and gentamicin was reported to be significantly more effective than either penicillin-vancomycin-gentamicin or penicillin-teicoplanin-gentamicin in the treatment of experimental penicillin- and glycopeptide-resistant *E. faecium* endocarditis (42). The authors postulated that ceftriaxone, which is normally ineffective against enterococci, must have some affinity for PBPs that become essential due to the induction of glycopeptide resistance. In all these cases, the strains expressed low-level aminoglycoside resistance; similar results may not be obtained with strains exhibiting high-level aminoglycoside resistance, since the addition of an aminoglycoside remains necessary for bacteriocidal activity. Other investigators did not obtain synergy with these regimens, leading to the conclusion that the phenomenon is strain dependent (45, 98, 118). Combinations involving double β -lactams have also been examined, and the combination of ampicillin and imipenem appeared to have a positive interaction in an experimental model of endocarditis in rabbits (34).

Ciprofloxacin and other quinolones introduced in the same period (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 (92, 222). Effectively, their use is limited to the treatment of urinary tract infections (183, 185). In a rat model of endocarditis, synergy with ciprofloxacin plus either gentamicin or rifampin or both was shown *in vitro* and *in vivo* against strains of vancomycin-resistant *E. faecium* lacking resistance to each antibiotic. Triple therapy (ciprofloxacin-rifampin-gentamicin) was the most effective at sterilizing vegetations (257). Although ciprofloxacin in high persistent concentrations may be effective in treating enterococcal endocarditis in the rat model, concentrations attainable in serum in humans have not yielded satisfactory results (73, 76, 92). In time-kill studies using strains of *E. faecium* with high-level resistance to ampicillin, vancomycin, and aminoglycosides, the combination of ampicillin at 40 $\mu\text{g/ml}$ plus ciprofloxacin at 3 $\mu\text{g/ml}$ was bactericidal for all strains with ciprofloxacin MIC of 8 $\mu\text{g/ml}$ or below. Unfortunately, regimens containing lower

concentrations of either antibiotic alone were not effective (150).

Newer fluoroquinolone antibiotics with greater activity against gram-positive bacteria have been created (207), and while enterococci remain among the least susceptible gram-positive bacteria (with *E. faecium* in general being less susceptible than *E. faecalis*), some compounds at 1 µg/ml or less inhibit 90% of strains (207). Clinafloxacin is the most active agent against enterococci among these new fluoroquinolones. The combination of ampicillin at 20 µg/ml with clinafloxacin at 1 µg/ml also had bactericidal activity against similar strains when the drugs were present in serum at concentrations that are easily attainable (38).

Novobiocin is an older DNA gyrase inhibitor with gram-positive activity. Clinical application of novobiocin was abandoned due to the emergence of resistance in staphylococci and the availability of newer, less toxic agents (200). In vitro data suggest that novobiocin is very active against VanA- or VanB-type vancomycin-resistant *E. faecium* strains, even if these strains are concomitantly resistant to penicillin and ampicillin (103). Novobiocin plus ciprofloxacin was found to be effective in a rabbit model of endocarditis (211). The combination of fluoroquinolones with novobiocin has also been investigated in other studies (103, 150, 211). Novobiocin was not bactericidal alone; however, upon addition of a fluoroquinolone, the combination was additive and bactericidal. The use of novobiocin combined with ciprofloxacin has been reported, with high relapse rates occurring after treatment (161). Resistance to quinolones, which is now common, would render such combinations ineffective because of the expected rapid emergence of resistance to novobiocin when used as a single agent. Nitrofurantoin is active against many isolates of VRE and might be an alternative for the treatment of urinary tract infections caused by VRE.

Treatment of multidrug-resistant enterococci that are resistant to penicillin, aminoglycosides, and glycopeptides is experimental at best (41, 128; B. M. Willey, D. Degani, and A. McGreer, Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 119, 1993). Currently, no prospective studies evaluating the efficacy of alternative drug regimens in infected patients have been published, although numerous investigators have studied various drugs and combinations both in vitro and in animal models. Dalfopristin-quinupristin (RP 59500) is a streptogramin antibiotic that has been studied in the treatment of infections due to vancomycin-resistant *E. faecium*. It has bacteristatic activity against strains of this species but is inactive against *E. faecalis*. It does not exhibit cross-resistance with any existing antibiotics (131, 250). In vitro studies have demonstrated increased activity of RP 59500 when combined with vancomycin against MRSA (136). However, no synergy was found against VRE when the drug was combined with ampicillin, ciprofloxacin, gentamicin, rifampin, streptomycin, teicoplanin, or vancomycin (L. B. Rice and L. L. Carias, Program Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E100, 1994). RP 59500 was first made available for use under an investigator-sponsored investigational new-drug program for treatment of patients with life-threatening infection due to vancomycin-resistant *E. faecium* (194). It is the first antibiotic approved for the treatment of patients with serious or life-threatening infections associated with vancomycin-resistant *E. faecium* bacteremia. In a recent review, Linden et al. compared the clinical and bacteriological outcomes of 20 patients with vancomycin-resistant *E. faecium* bacteremia treated with RP 59500 with a historical cohort of 42 patients with VRE bacteremia treated with other agents (162). They found that despite the high overall mortality rates in both

groups, quinupristin-dalfopristin therapy was associated with a significantly lower incidence of vancomycin-resistant *E. faecium*-associated mortality. On the other hand, frank clinical failure was seen in five quinupristin-dalfopristin-treated patients. One failure occurred in a patient with refractory neutropenia following drug-induced bone marrow suppression. The lack of bactericidal activity of quinupristin-dalfopristin may compromise its clinical and bacteriological efficacy in neutropenia and other conditions where bactericidal activity is required for eradication (174). However, satisfactory outcomes have been reported in other challenging clinical conditions. Quinupristin-dalfopristin therapy achieved microbiological and clinical cure in a patient with vancomycin-resistant *E. faecium* prosthetic valve endocarditis, in an 8-month-old infant with ventriculitis due to a vancomycin-resistant *E. faecium*-infected central nervous system shunt and in three cases of vancomycin-resistant *E. faecium* peritonitis due to peritoneal dialysis catheter-associated infection (166, 187; W. B. Furlong and F. Bompert, Program Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M66, 1994). Superinfection with *E. faecalis* has been observed during therapy of *E. faecium* infection with this agent (49). De novo resistance to quinupristin-dalfopristin among *E. faecium* strains has been reported previously (55), and the rise in MICs of quinupristin-dalfopristin to 1 to 2 µg/ml in the study by Linden et al. raises the possibility that frank resistance to quinupristin-dalfopristin could develop in some strains. An increase in the MIC of the drug associated with relapse after therapy has also been observed (50, 161).

There has been a 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. Its MICs were low in most investigations (26, 48, 170, 234), with strains of all species of *Enterococcus* being inhibited by 8 µg/ml and MICs at which 90% of isolates were inhibited (MIC₉₀s) being recorded up to 4 µg/ml; glycopeptide-resistant *E. faecium* strains were included (25, 166). The inoculum effect was small, the agent was effectively bactericidal alone (unlike teicoplanin and vancomycin) (25, 261), and there was no cross-resistance with vancomycin and teicoplanin. Addition of serum to the medium impaired the in vitro activity (25, 157, 170, 240). Variable but quite encouraging results were obtained in animal models. Although daptomycin was well tolerated, the early results in clinical trials were disappointing; the drug was ineffective for the treatment of staphylococcal septicemia (108). This may have been the result of the very high (>90%) plasma protein binding, and a higher dosage might have provided effective therapy (148, 220). Unfortunately, higher-dosage regimens were associated with toxicity in humans, and the drug was withdrawn in 1990 (18).

Ramoplanin, a lipoglycopeptide, is even more active. In all investigations, all strains of all species, including vancomycin-resistant *E. faecalis* and *E. faecium*, were inhibited by 8 µg/ml and the MIC₉₀s (in all but one study) ranged from ≤0.25 to 1.6 µg/ml (18, 54, 132, 170, 234). Ramoplanin is also bactericidal for enterococci, with the MBCs being only fourfold higher than the MICs (132). Ramoplanin inhibits cell wall synthesis by acting at the level of lipid-intermediate formation, whereas vancomycin and daptomycin interfere with peptidoglycan synthesis by preventing transglycosylation (223). Addition of human serum results in fourfold increase in the MIC. In preliminary studies, ramoplanin has been poorly tolerated following intravenous or intramuscular injection, and it seems unsuitable for systemic use because of its toxicity; however, it is being developed for topical use (261). It has been suggested

that ramoplanin could be used for the clearance of glycopeptide-resistant enterococci from the gastrointestinal tract (132), and it might well be used to eradicate *C. difficile* without a risk of colonization by glycopeptide-resistant enterococci (22).

One of the most active agents against VRE is a semisynthetic glycopeptide designated LY333328, which demonstrates bactericidal as well as bacteristatic activity against enterococci (229). LY333328 is an investigational *N*-alkyl semisynthetic derivative of the naturally occurring glycopeptide LY264826 (56). Its mechanism of action is still unknown but is thought to be similar to that of vancomycin. The primary mechanism appears to be the inhibition of cell wall synthesis and assembly by complexing with the D-alanyl-D-alanine precursor. It might also impair RNA synthesis (90, 171). Several studies have shown that LY 333328 exhibits bactericidal activity against VRE (202; S. Zelenitsky, J. Karlowsky, D. Hoban, et al., Program Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F200, 1996). The reported MIC₉₀s for different strains of MRSA and VRE (VanA and VanB strains) are ≤ 1 μ g/ml. Overall, LY333328 has been reported to exhibit a higher rate of killing and a higher concentration-dependent killing effect against both staphylococci and enterococci (M. S. Barret, M. E. Erwin, and R. N. Jones, Program Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F203, 1996; S. Donabedian, M. B. Perri, L. A. Thal, and M. J. Zervos, Program Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F204, 1996; Zelenitsky et al., 36th ICAAC). Concentration-dependent killing is a unique finding, since it has not been previously reported for glycopeptides (13, 90, 169). This effect is significant, because LY333328 is affected by proteins and inoculum and so a slight increase in drug concentration can easily compensate for these effects. The lack of data regarding the pharmacokinetics in humans and the toxicity of LY333328 creates uncertainties about whether it is possible to achieve the desired concentrations in humans that would compensate for the inoculum effect and protein binding without causing serious side effects (169). Such a problem has been described in the past with daptomycin. The excellent inhibitory and bactericidal activities of LY333328 suggest that it could be a clinically useful alternative for the treatment of severe infections caused by gram-positive pathogens (including VRE), particularly those resistant or not fully susceptible to the available glycopeptides (23). In preliminary in vivo studies, LY333328 has been reported to have a considerable advantage over vancomycin in terms of its pharmacokinetics, with apparently a much longer half-life in rats (Y. Lin, R. E. Stratford, L. L. Zornes et al., Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F254, 1995). In two recent studies, it was found that the postantibiotic effect for LY333328 was prolonged (18.7 h at 10 times the MIC and 7.4 ± 2.2 h at 10 times the MBC) (14; A. Novelli, T. Mazzei, S. Fallani, et al., Program Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-16, p. 148, 1997). In one of these studies, it was also shown that the presence of ampicillin (10 times the MIC) increased the PAE to 23 h and that ampicillin, quinipristin-dalfopristin, and gentamicin exhibited bacteriostatic synergy with LY333328 (14). However, 50% serum decreased the postantibiotic effect by 30 to 50%. These data also indicate the persistence of the effect of LY333328 for many hours and therefore the possibility that therapy may require infrequent dosing.

Other peptide antimicrobial agents have been investigated for activity against enterococci. Derivatives of vancomycin may show increased activity against enterococci, including glycopeptide-resistant strains (191), but development of these compounds is at an early stage. There are also derivatives of teico-

planin, LY264826, and other glycopeptides with increased antienterococcal activity (39, 190, 191, 224, 236, 264). Some of them are not bactericidal, some show cross-resistance with teicoplanin- and vancomycin-resistant strains, and they are still at early stages of development. 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 glycopeptide that is effective and nontoxic in systemic use and lacks cross-resistance with vancomycin and teicoplanin (91).

Other investigational agents with activity in vitro against VRE include glycyclines, oxazolidinones, and ketolides. Some isolates of VRE are susceptible to tetracyclines. Doxycycline and minocycline have been used in the treatment of VRE infections, often with other agents. While successes have been described, it is difficult to assess their overall effectiveness (47, 176). The high rate of tetracycline resistance, especially among clinically important gram-positive cocci, has limited its effectiveness (77, 239). Tetracycline derivatives known as glycyclines have excellent activity against enterococci including multidrug-resistant strains. The glycyclines CL329,998 (DMG-MINO) and CL331,002 (DMG-DMDOT) are *N,N*-dimethylglycylamido derivatives of 9-aminomincycline and 9-amino-6-demethyl-6-deoxytetracycline, respectively. These compounds were developed to overcome the three types of resistance mechanisms exhibited by many clinically important bacteria and to restore a broader range of activity to the tetracycline class. DMG-MINO and DMG-DMDOT show excellent activity against strains resistant to tetracycline due to a ribosomal protection mechanism and to the production of efflux pumps (245). Thus, modification at position 9 overcomes two distinct resistance mechanisms. These drugs show activity against most enterococcal strains, including those resistant to parent drugs or to other antimicrobial agents (74). Their activity remains bacteristatic. Preclinical toxicological studies have shown qualitatively similar profiles to those noted with minocycline and tetracycline (243). If they are effective clinically, the glycyclines will be important drugs for the treatment of infections caused by resistant gram-positive bacteria, but they are still a long way from being marketed (99, 243).

Ketolides are a new class of macrolide derivatives with good gram-positive activity (C. Agouridas, Y. Beneditti, A. Bonnefoy, et al., Program Abstr. 3rd Int. Conf. Macrolides Azalides Streptogramins, abstr. F14.10, 1996; C. Agouridas, A. Bonnefoy, and J. F. Chantot, Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F158, 1995). Instead of the cladinose moiety, these agents have a 2-keto structure, which appears to increase their stability in a weakly acidic environment (Agouridas et al., 3rd Int. Conf.). Their mechanism of action is similar to that of macrolides or macrolide-lincosamide-streptogramin compounds, which consists of binding to the 50S ribosomal subunit and inhibition of bacterial protein synthesis (C. Agouridas, A. Bonnefoy, K. Braham, et al., Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F175, 1995). They also penetrate well into phagocytes (C. Agouridas, P. Coletto, P. Mauvais, and J. F. Chantot, Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F170, 1995). Ketolides show in vitro activity against multidrug-resistant gram-positive organisms, including staphylococci, enterococci and pneumococci (L. M. Ednie, S. K. Spaangler, M. R. Jacobs, and P. C. Appelbaum, Program Abstr. 3rd Int. Conf. Macrolides Azalides Streptogramins, abstr. F3.19, 1996; A. Fremaux, G. Sissia, J. F. Chantot, and P. Geslin, Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F160, 1995). RU-64004 is a novel ketolide. For vancomycin-resistant *E. faecium* strains,

the potential spectrum of RP 59500 was found to be equal or superior to that of RU-64004 (130; Ednie et al., 3rd Int. Conf.; Fremaux et al., 35th ICAAC). In vivo studies with animal models have shown quite promising results, especially against infections caused by macrolide-resistant strains of gram-positive bacteria, although there are at present few data on their antienterococcal activity (Agouridas et al., 35th ICAAC, abstr. F158). Results with another ketolide, RU-66647, are similar to earlier data reported for RU-64004 (130; Agouridas et al., 3rd Int. Conf.; Agouridas et al., 35th ICAAC, abstr. F175 and F170; H. Dabernat, M. Seguy, and C. Delmas, Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F161, 1995; Ednie et al., 3rd Int. Conf.; R. Fabre, J. D. Cavallo, J. C. Chapalain, and M. Meyron, Program Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F164, 1995; Fremaux et al., 35th ICAAC).

The oxazolidinones are a new class of synthetic antibiotics with good antienterococcal activity and are different from any other class (75). The mechanism of activity demonstrated has been the inhibition of protein synthesis leading to a bacteriostatic effect against most species (62). The oxazolidinones inhibit bacterial translocation at the initiation of protein synthesis (159, 233). It appears from previous studies that in vitro selection of resistant mutants does not occur readily (62). Mechanisms of resistance that affect antibiotics in current clinical use do not affect the activities of oxazolidinones. The oxazolidinones are active when given orally. Eperzolid and linezolid are two investigational oxazolidinone agents which are in phase II and phase III clinical trials, respectively. They show excellent activity against multiantibiotic-resistant enterococci characterized by low MICs (28). Clinical efficacy and safety studies are needed to determine their ultimate utility. Linezolid has recently been approved by the Food and Drug Administration (FDA).

Rifampin alone has very limited usefulness in the treatment of enterococcal infections (172) because of its poor bactericidal activity and because of the presence and the emergence of subpopulations of resistant bacteria, both in vitro and in vivo (172). Since rifampin remains active against many strains of multiresistant enterococci, it is often tested in combination with other agents.

Fosfomycin has activity against enterococci (116), but rapid emergence of resistance limits its usefulness as a single agent.

Due to lack of data concerning the treatment of multidrug-resistant enterococcal infections, it is probably prudent to test potential drug combinations in vitro using time-kill curves and apply the results to modify therapy. Checkerboard synergy panels are not recommended, since the results of these tests do not consistently correlate well with bactericidal activity in test tube-killing curves (153).

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