# Emergence of Vancomycin-Resistant Enterococci

**Louis B. Rice**
VA Medical Center and Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

Vancomycin and ampicillin resistance in clinical *Enterococcus faecium* strains has developed in the past decade. Failure to adhere to strict infection control to prevent the spread of these pathogens has been well established. New data implicate the use of specific classes of antimicrobial agents in the spread of vancomycin-resistant enterococci (VRE). Extended-spectrum cephalosporins and drugs with potent activity against anaerobic bacteria may promote infection and colonization with VRE and may exert different effects on the initial establishment and persistence of high-density colonization. Control of VRE will require better understanding of the mechanisms by which different classes of drugs promote gastrointestinal colonization.

Enterococci are important nosocomial pathogens (1,2). Their emergence in the past two decades is in many respects attributable to their resistance to many commonly used antimicrobial agents (aminoglycosides, aztreonam, cephalosporins, clindamycin, the semi-synthetic penicillins nafcillin and oxacillin, and trimethoprim-sulfamethoxazole) (3). Exposure to cephalosporins is a particularly important risk factor for colonization and infection with enterococci (4-6). Thus, the era in which safe and effective cephalosporins became widely available has also been an era of enterococcal ascendance.

## Ampicillin Resistance

Ampicillin is the therapy of choice for enterococcal infections. Ampicillin MICs for *Enterococcus faecalis*, the most commonly isolated enterococcal species from clinical cultures, generally are 0.5 to 4.0 µg/mL, whereas for the less commonly isolated *E. faecium*, MICs are 4 to 8 µg/mL. *E. faecalis* and *E. faecium* account for >95% of enterococcal isolates from clinical cultures. Low-level ampicillin resistance in enterococci is attributable to the production of a low-affinity penicillin-binding protein (PBP), PBP 5 (7). PBP 5s have been identified in several enterococcal species. Those of *E. faecalis*, *E. faecium*, and the closely related *E. hirae* demonstrate <75% nucleic acid identity, but the fact that antibodies raised against one bind to all three suggests substantial structural similarity (8).

Increased ampicillin resistance in enterococci is attributable to either the production of beta-lactamase or alterations in the expression or structure of PBP 5. Beta-lactamase production has been described almost exclusively in *E. faecalis* and is attributable in most cases to the acquisition of the *Staphylococcus aureus* beta-lactamase operon (9-11). Beta-lactamase production occurs at a low level in enterococci, conferring a minor increase in MIC at standard inoculum. MIC increases more dramatically at high inoculum, however, and animal studies suggest that expression of this determinant may affect the outcome of endocarditis (12).

Ampicillin resistance resulting from changes in PBP 5 is primarily a clinical problem in *E. faecium*. The first detailed information about PBP 5-mediated ampicillin resistance arose from several lines of investigation. Williamson et al. noted that penicillin resistance expressed by *E. faecium* was related to the amount and the affinity of PBP 5 (13). The observation that enterococci could grow normally in penicillin concentrations enough to saturate all the PBPs, except PBP 5, suggested that PBP 5 was capable of carrying out all the functions necessary for cell-wall synthesis. Eliopoulos et al. derived a hypersusceptible mutant of a clinical *E. faecium* strain and noted that it no longer produced detectable amounts of PBP 5 (14). Subsequent studies confirmed that the lack of PBP 5 expression in this mutant was due to loss of the *pbp5* gene (15). Fontana et al. described in vitro mutants of *E. hirae* 9790 that expressed increased levels of resistance to ampicillin (MIC 64 µg/mL) (16). These mutants were found to produce increased quantities of PBP 5. In the initially analyzed strain, increased PBP 5 production was associated with a deletion within an upstream open reading frame that was characterized as a penicillin-binding protein synthesis repressor (psr) (17). A more recent study suggests that psr may serve as a global regulator of cell-wall synthesis genes in enterococci (18).

*E. faecium* strains expressing very high levels of ampicillin resistance (MIC >128 µg/mL) emerged in U.S. medical centers in the late 1980s (19). Molecular analysis of these strains suggested that the increase was attributable to mutations within the *pbp5* gene, which decreased the binding affinity of PBP 5 for ampicillin (20,21). One clinical study associated colonization with ampicillin-resistant *E. faecium* and prior therapy with extended-spectrum cephalosporins (22).

During the late 1980s, the prevalence of methicillin-resistant staphylococci was also increasing in U.S. hospitals (1), resulting in increased use of vancomycin. The discovery that antibiotic-associated diarrhea and pseudomembranous colitis were due to *Clostridium difficile* further fueled vancomycin use (23).

Address for correspondence: Louis B. Rice, Medical Service 111(W),VA Medical Center, 10701 East Blvd., Cleveland, OH 44106, USA; fax: 216-231-3289; e-mail: louis.rice@med.va.gov

## Vancomycin Resistance

Vancomycin-resistant enterococci (VRE) were first reported in 1986, nearly 30 years after vancomycin was clinically introduced. The primary inciting factor was likely the use of orally administered vancomycin for treating antibiotic-associated diarrhea in hospitals. Vancomycin resistance is conferred by one of two functionally similar operons, VanA or VanB (Figure) (24). The VanA and VanB operons are highly sophisticated resistance determinants, which suggests that they evolved in other species and were acquired by enterococci. The difference in the guanine-cytosine (G-C) content of the genes of the VanB operon (roughly 50% G-C) (25) in comparison to typical enterococcal genes (35% to 40% G-C) (3) is compelling evidence for this acquisition. The conditions that would favor substantial colonization by naturally glycopeptide-resistant species (probably streptomycetes) and persistence of enterococci include high vancomycin concentrations in the gastrointestinal tract. Substantially high levels of glycopeptides in the gastrointestinal tract are achievable by oral administration, since these agents are not absorbed, resulting in fecal vancomycin concentrations high enough to favor colonization with vancomycin-resistant streptomycetes, but not high enough to kill the notably tolerant enterococcus. Hence, it is reasonable to presume that oral administration of glycopeptides to humans was a major factor in the emergence of vancomycin resistance in enterococci. The European VRE outbreak's apparent origin in animals (who were fed oral glycopeptides as growth promoters) further supports this scenario.

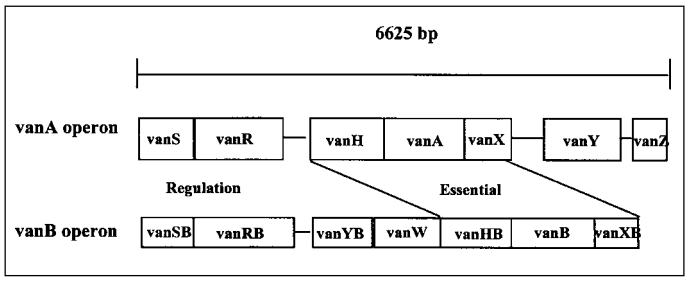


Figure. Comparison of arrangements of the VanA and VanB glycopeptide resistance operons. Essential genes and those involved in regulation of expression of the resistance determinant are marked.

## Risk Factors for Multidrug-Resistant Enterococci

More than 95% of VRE recovered in the United States are *E. faecium*; virtually all are resistant to high levels of ampicillin. The phenotypic association of ampicillin and vancomycin resistance is in some instances due to genetic linkage. We reported transferable ampicillin and VanB-type vancomycin resistance from *E. faecium* strains isolated in northeast Ohio (26). Both *pbp5* and the *vanB* operon were located in the chromosome and linked as a result of the insertion of a VanB transposon (Tn*5382*) immediately downstream of *pbp5* (15). Both determinants were located within a larger mobile element that was able to transfer between *E. faecium* strains. This larger transposon is widely disseminated; it is found in clonally unrelated *E. faecium* isolates from New York, Pennsylvania, Florida, Missouri, Ohio, and Hawaii (27).

*E. faecium* is less pathogenic than *E. faecalis*; in fact, many VRE infections resolve without active antimicrobial-drug therapy (28). However, in specific patient populations, notably in liver transplant patients and patients with hematologic malignancies, VRE cause serious and often fatal disease (29,30). Therefore, it is well worth understanding the factors that promote the emergence and spread of multidrug-resistant VRE.

Frequently identified risk factors for VRE colonization and infection include prolonged hospital stays, exposure to intensive care units, transplants, hematologic malignancies, and exposure to antibiotics (31). The epidemiology of VRE spread in the hospital involves both person-to-person transmission and selective antibiotic pressure. Very specific practices designed to prevent the person-to-person spread of VRE have been recommended by the Hospital Infection Control Practices Advisory Committee to the Centers for Disease Control and Prevention and are in place in many hospitals (32). These measures include surveillance for colonization, identification of colonized and infected patients, isolation or cohorting of colonized persons, strict use of gloves and gowns by people coming into contact with the patient, thorough room cleaning after patient discharge, and efforts to limit use of vancomycin in hospitals. In geographically limited outbreaks caused by the dissemination of a single VRE clone, these practices have successfully eliminated the organisms from the hospital (33-35). In larger, more disseminated outbreaks caused by several different VRE clones, infection control measures and control of vancomycin use have shown only limited efficacy, suggesting selection pressure by antimicrobial drugs other than vancomycin (36,37).

Antibiotics other than glycopeptides have been linked with increased risk for colonization and infection with VRE, most prominently, the extended-spectrum cephalosporins and antibiotics with potent activity against anaerobic bacteria (26,31,38,39). These associations have been noted in retrospective, uncontrolled studies.

## Nonglycopeptide Antibiotics and VRE

Are there compelling reasons to believe that cephalosporins or antibiotics with potent activity against anaerobic bacteria increase risk for VRE? Early studies reported VRE strains in which exposure to vancomycin increased the susceptibility to beta-lactams (40). It was hypothesized that PBP 5 was unable to process peptidoglycan precursors terminating in D-lactate. Therefore, expression of vancomycin resistance, whose mechanism in both VanA and VanB strains involves the substitution of D-lactate for D-alanine at the terminus of the pentapeptide precursors, would need to involve other PBPs in cell-wall synthesis. These other PBPs would be susceptible to beta-lactams, including cephalosporins. However, mutants resistant to synergism are relatively easy to select in vitro, and strains resistant to such synergism are commonly found in the clinical setting (41).

The cephalosporin association may be related to the fact that virtually all VRE in the United States express high-level ampicillin resistance. The high-level ampicillin-resistant strains express even higher degrees of resistance to extended-spectrum cephalosporins (>10,000 µg/mL) (26). The concentrations of cephalosporins achievable in bile (as high as 5,000 µg/mL for ceftriaxone) (42-44) can inhibit or kill virtually all upper gastrointestinal bacterial flora, except for VRE. On the

other hand, antienterococcal penicillins such as piperacillin, which appear to be protective against VRE in some clinical studies, achieve biliary concentrations in excess of 1,000 µg/mL in human bile after standard doses (45). These concentrations exceed the MIC of most VRE for piperacillin (256 to 1024 µg/mL). It is therefore within reason that the potentially protective effect observed with piperacillin is explainable by its direct inhibition of VRE in the upper gastrointestinal tract.

We tested this hypothesis in an animal model in which subcutaneous doses of different antimicrobial agents were administered to mice for 2 days, followed by intragastric injection of small numbers (ca. 100 CFU) of a highly ampicillin-resistant VRE strain B *E. faecium* C68 (46). Stool samples were subsequently collected over a 2- to 3-week period to determine whether high-level VRE colonization was established. In this model, subcutaneous administration of piperacillin-tazobactam was found to protect against high-level VRE colonization, whereas ceftriaxone and ticarcillin-clavulanic acid (with antienterococcal activity equivalent to the cephalosporins) promoted high-level VRE colonization (Table 1). These results are consistent with a model in which piperacillin is protective because of direct inhibition of VRE in the upper gastrointestinal tract, whereas ceftriaxone and ticarcillin promote colonization because they inhibit everything but VRE, thereby permitting high-level colonization.

Table 1. Pretreatment with antibiotics and vancomycin-resistant enterococci (VRE) colonization after gastric administration of $10^2$ CFU vancomycin and ampicillin-resistant *Enterococcus faecium* C68 (46)

| | Approximate $\log_{10}$ CFU VRE/g stool | | | | |
|---|---|---|---|---|---|
| | Day 3 | Day 6 | Day 9 | Day 13 | Day 16 |
| Saline | 2 | 2.5 | 3 | 2.5 | 2.5 |
| Piperacillin-tazobactam | 2 | 2 | 2 | 2 | 2 |
| Ticarcillin-clavulanic acid | >9 | >9 | 8.2 | 6.8 | 6.8 |
| Ceftriaxone | >9 | 8.8 | 8.4 | 7.2 | 6 |

A direct activity of antianaerobic antibiotics against VRE is more difficult to understand, since some of these antibiotics are among the most active antienterococcal agents (ampicillin-sulbactam, piperacillin-tazobactam), and most of the extended-spectrum cephalosporins have relatively weak activity against anaerobes. Conceivably, however, these antibiotics exhibit potent activity against species that successfully compete with enterococci for colonization of the gastrointestinal tract, thereby promoting persistence of high-level VRE colonization once it is successfully established. We tested this hypothesis in a separate animal model in which high-level VRE colonization was established by intragastric injection of $10^6$ CFU of C68 after administration of oral vancomycin (47). This technique established colonization of mouse stool with $10^9$ CFU of VRE in all animals. When oral vancomycin was discontinued, colonization levels declined at a regular and predictable rate; most animals had no detectable colonization after 3 weeks. We tested the effects of subcutaneous administration of different antibiotics on the persistence of high-level VRE colonization (Table 2). Vancomycin and antibiotics with potent activity against anaerobic bacteria (ampicillin-sulbactam, cefoxitin, clindamycin, metronidazole, piperacillin-tazobactam, and ticarcillin-clavulanic acid) promoted persistence of high-level VRE colonization, even though some had excellent activity against enterococci and had been shown to prevent VRE colonization in the other model (see above). In contrast, antibiotics with relatively poor antianaerobic activity (aztreonam, cefepime, ceftriaxone, ciprofloxacin) did not promote high-level colonization.

Table 2. Antibiotic treatment and persistence of high-level colonization with vancomycin and ampicillin-resistant *Enterococcus faecium* C68 (47)

| | Approximate $\log_{10}$ CFU VRE/g stool[a] | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 4-5 | Day 9-10 | Day 14-15 | Day 19-20 |
| Saline | 9.5 | 8.3 | 6 | 3.8 | 3.5 |
| Vancomycin (SQ) | >9 | >9 | >9 | >9 | >9 |
| Vancomycin (oral) | >9 | >9 | >9 | >9 | >9 |
| Antibiotics with potent antianaerobic activity | | | | | |
| Piperacillin-tazobactam | >9 | >9 | >9 | >9 | >9 |
| Ticarcillin-clavulanic acid | >9 | >9 | >9 | >9 | >9 |
| Clindamycin | >9 | >9 | >9 | >9 | >9 |
| Cefotetan | >9 | >9 | 8.8 | 7.8 | 8 |
| Metronidazole | >9 | >9 | >9 | >9 | >9 |
| Ampicillin | >9 | >9 | 8 | 7.2 | 7 |
| Ampicillin-sulbactam | >9 | >9 | >9 | 7.8 | 7.7 |
| Antibiotics with relatively poor activity against anaerobic bacteria | | | | | |
| Cefepime | >9 | >9 | 6.2 | 5 | 4.8 |
| Ceftriaxone | >9 | 8.8 | 8.4 | 7.2 | 6 |
| Aztreonam | >9 | 9 | 4.3 | 4.2 | 3.8 |
| Ciprofloxacin | >9 | 8.8 | 6 | 5.2 | 5 |

[a]VRE = vancomycin-resistant enterococci; SQ = subcutaneous.

## Antibiotics and VRE Colonization and Infection

The above results suggest a model for antibiotic influence on the spread of VRE. Commonly used antibiotics that achieve high gastrointestinal concentrations but are inactive against enterococci, such as the cephalosporins, ticarcillin, and perhaps vancomycin, favor colonization with high levels of VRE in the stool. Antibiotics active against anaerobic bacteria, which are the primary competitors of enterococci for colonizing the gastrointestinal tract, favor the persistence of high levels of VRE in stool but may or may not (depending on their intrinsic antienterococcal activity) favor colonization in uncolonized patients. Antibiotics that meet both criteria, such as ticarcillin-clavulanic acid, should be particularly associated with VRE. In a citywide analysis of hospitals in the greater Cleveland area, the use of ticarcillin-clavulanic acid was associated with higher hospital rates of clinical VRE (26). A positive, although not statistically significant, association was noted for extended-spectrum cephalosporins, while a negative but statistically insignificant association was noted for the combination of ampicillin, ampicillin-sulbactam, piperacillin, and piperacillin-tazobactam.

The frequent association of cephalosporins with VRE colonization and the failure to associate piperacillin-tazobactam with VRE suggest that the most important

driving force for the emergence and spread of these organisms within institutions may be the predilection for establishing new colonizations. This is not to say that antimicrobial agents that promote persistence of high-level colonization will not be important for promoting VRE outbreaks, but that this effect is less pronounced if high-volume use of cephalosporins (or ticarcillin-clavulanic acid) does not create receptive new environments for establishing new colonization.

These data also suggest that refined strategies can be developed to limit the emergence and spread of VRE within hospitals. Commitment to serious infection control practices and limitation of vancomycin use must remain the cornerstones of any successful strategy. However, it is possible to envision settings where surveillance-culturing systems are taken seriously and patients who are colonized with VRE are routinely identified. In such settings, the choice of which empiric antibiotic to administer for a presumed nosocomial infection would be affected by the colonization status of the patient. In patients known to be colonized with VRE, broad-spectrum agents that lack significant activity against anaerobes (such as extended-spectrum cephalosporins of fluoroquinolones) would be preferred, on the assumption that potent anaerobic activity would not be required for treating the infection. If the patient is not colonized with VRE, administration of a potent antienterococcal broad-spectrum agent such as piperacillin-tazobactam may be preferred. In this manner, both the establishment of new colonization and the level of colonization of those already colonized could be minimized.

## Conclusions

Multidrug-resistant enterococci continue to pose problems in U.S. medical centers. The best available evidence suggests that the emergence and spread of these pathogens are promoted by poor infection control techniques and by antibiotic selective pressure. Antibiotic selective pressure favoring the emergence and spread of VRE may involve more than simply the extent of vancomycin use. Specifically, extended-spectrum cephalosporins and similarly active beta-lactams and drugs with potent activity against anaerobes appear to predispose to VRE colonization and infection. On one hand, data from animal models suggest that the cephalosporins predispose to establishment of VRE colonization through their potent activity against many bacteria and essential lack of activity against ampicillin-resistant enterococci. On the other hand, antianaerobic antibiotics appear to favor persistence of high levels of VRE colonization through their activity against competing flora. A more detailed understanding of the impact of different antibiotics on the upper and lower gastrointestinal flora will be an important step in controlling the emergence and spread of VRE.

Dr. Rice is chief of the medical service at the Louis Stokes Cleveland Veterans Administration Medical Center, vice chairman of the department of medicine at University Hospitals of Cleveland, and professor of medicine at Case Western Reserve University. His primary research interests are in the mechanisms of antimicrobial resistance and resistance transfer in enterococci and the evolution of extended-spectrum beta-lactamases in gram-negative bacilli.

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