

# Vancomycin-Resistant Enterococci

Roberto C. Arduino, M.D.\*

Barbara E. Murray, M.D.\*

## INTRODUCTION

Enterococci have long been recognized as pathogenic organisms able to cause endocarditis, bacteremias, infections of the urinary and biliary tracts, skin and soft-tissue infections and meningitis in neonates and after neurosurgical procedures (66). Their pathogenic role in wound infections, intraabdominal abscesses, diabetic foot and decubitus ulcers, however, is more controversial since enterococci are frequently part of a polymicrobial flora and since patients with these infections often recover despite therapy with regimens without specific activity against enterococci (53). Between 80-95% of the enterococcal infections are caused by *Enterococcus faecalis* and 5-15% by *Enterococcus faecium*. Although there are increasing reports of infections caused by *Enterococcus durans*, *Enterococcus avium*, *Enterococcus raffinosus*, *Enterococcus gallinarum*, and *Enterococcus casseliflavus*, these species are still isolated infrequently from human sources (53).

Enterococci are now the second most common hospital-acquired pathogens in the United States. The most recent National Nosocomial Infections Surveillance (NNIS) system report from the Centers for Disease Control and Prevention indicated that enterococci were isolated from 12% of all nosocomial infections from 1986 to 1989 (63). The majority were urinary tract infections. The relative resistance to penicillins, intrinsic low-level resistance to aminoglycosides and intrinsic resistance to a number of other antimicrobial agents, particularly the cephalosporins which are heavily used in hospitalized patients, may

explain the emergence of enterococci as significant nosocomial pathogens.

In the past two decades enterococci have acquired resistance to a number of clinically important antimicrobial agents making them more difficult to treat, particularly when a bactericidal effect is required such as for endocarditis and meningitis. These resistances include high-level resistance to aminoglycosides (MIC > 2000 µg/ml), which abrogates the synergistic killing effect seen when these agents are combined with penicillin; resistance to the B-lactams, by production of B-lactamase or penicillin binding proteins (PBPs) with low affinity for these antibiotics; and most recently, resistance to vancomycin. Initially reported in England and France in the late 1980's (29,71), enterococci with various levels of resistance to vancomycin and teicoplanin are now reported with increasing frequency from all over the world (29,33,37,39,40,47,49). The NNIS system recently reported that the percentage of vancomycin-resistant enterococci associated with nosocomial infections in the United States increased from 0.3% in 1989 to 7.9% in 1993 (13). Approximately 14% of enterococci isolated from patients with nosocomial infections in intensive care units from hospitals included in the NNIS survey were resistant to vancomycin. A recent study that recruited 99 microbiology laboratories in the United States reported an incidence of 4.4% resistance to vancomycin and 2.2% of combined high-level resistance to aminoglycosides, ampicillin and glycopeptides among 1,936 enterococcal isolates (38).

Vancomycin-resistant enterococci are most frequently isolated from gastrointestinal (e.g., abdominal abscess),

\*From the Departments of Internal Medicine and Microbiology and Molecular Genetics, and Center for Infectious Diseases, The University of Texas Medical School, Houston, Texas 77030

Received for publication : March 28, 1994

Reprint request : Professor Barbara E. Murray, M.D., Center for Infectious Diseases, The University of Texas Medical School, Houston, Texas 77030

**Key words :** Enterococci, vancomycin, drug-resistance, glycopeptide resistance, vancomycin resistance

skin and soft tissue, and bloodstream sites in hospitalized patients (13,38). Multiple or prolonged courses of antibiotics, including vancomycin, and severe underlying medical conditions, such as end-stage renal disease, immunosuppressive therapy or hematologic malignancies, are predisposing factors frequently reported among patients colonized or infected with these resistant organisms (27,29,33,39,44,48,51,60,71,72,73).

Vancomycin and teicoplanin are glycopeptides with a narrow-spectrum of activity against many aerobic and anaerobic gram-positive bacteria. Minimal inhibitory concentrations (MICs) of vancomycin for enterococci usually range from 0.5 to 8.0  $\mu\text{g/ml}$  (41,68), and those of teicoplanin from 0.12 to 0.5  $\mu\text{g/ml}$  (19). Like other cell-wall active antimicrobials, glycopeptides are typically bacteriostatic against enterococci and the minimal bactericidal concentration (MBC) is generally more than 100-fold higher than the concentration required to inhibit the bacterial growth; that is, enterococci are tolerant to cell-wall active antibiotics (34). Moreover, time-kill curves often show a slower rate of killing with vancomycin alone than with ampicillin alone (19). However, vancomycin- or teicoplanin-aminoglycoside combinations are usually synergistic as long as the organism does not possess high-level resistance to the aminoglycoside (77).

### Vancomycin Resistance : Classification

Three major phenotypes of glycopeptide resistance were initially distinguished among enterococci : VanA phenotype is associated with inducible high-level resistance to vancomycin and teicoplanin, VanB phenotype with inducible resistance to vancomycin only, and VanC

phenotype with constitutive resistance to low levels of vancomycin only. These resistance phenotypes, however, do not always correspond with the resistance genotypes since *vanA*-containing isolates can exhibit MICs of vancomycin overlapping those of *vanB*-containing isolates (e.g., 64  $\mu\text{g/ml}$ ) and *vanB*-containing isolates can exhibit high-level resistance to vancomycin and even resistance to teicoplanin. Furthermore, *E. gallinarum* and *E. casseliflavus* exhibit constitutive low-level resistance to vancomycin but their resistances are conferred by different genes. Therefore, the classification presented here is based on the presence of the genes *vanA*, *vanB*, and *vanC* (Table 1).

Enterococci containing *vanA* typically have high-level resistance to vancomycin (MICs,  $\geq 64\mu\text{g/ml}$ ) and teicoplanin (MICs,  $\geq 16\mu\text{g/ml}$ ). Exposure of these organisms to either of these glycopeptides induces the production of two new cytoplasmic membrane-associated proteins: a 39-kDa D-alanine: D-alanine ligase termed VanA, that is necessary for the synthesis of cell-wall precursors in the presence of glycopeptides, and a D,D-carboxypeptidase termed VanY, that is not required for glycopeptide resistance (2,4,30,55). A cluster of nine genes conferring high-level resistance to vancomycin and teicoplanin was recently found within a transposon designated Tn1546 which is carried on a plasmid (3,5). The products of this gene cluster include: a transposase, a resolvase, VanR, VanS, VanH, VanA, VanX, VanY and VanZ. The transposase and the resolvase are involved in the mobilization of the transposon. VanR and VanS comprise a two-component signal-transducing system that activates a promoter for

Table 1. Classification and relevant features of vancomycin-resistant enterococci.

Phenotype	MIC( $\mu\text{g/ml}$ )		Induced Membrane Protein		Location of resistance	Induction		Bacterial species
	Vancomycin	Teicoplanin	Protein(s)	Activity		Vancomycin	Teicoplanin	
VanA	$\geq 64$	$\geq 16$	VanA	D-alanine ligase	Transposon (Tn1546)	+	+	<i>E. faecium</i> <i>E. faecalis</i> <i>E. avium</i>
			VanY	D,D-carboxypeptidase				
VanB	8-1024	$\leq 1^a$	VanB	D-alanine ligase	Unknown but can be transferred	+	-	<i>E. faecalis</i> <i>E. faecium</i>
VanC	8-32	$\leq 1$	VanC	D-alanine ligase	Chromosomal	-	-	<i>E. gallinarum</i>
"VanC-like"	8-32	$\leq 1$	-	-	Chromosomal	-	-	<i>E. casseliflavus</i>

<sup>a</sup>Teicoplanin-resistant mutants can arise *in vivo* and *in vitro*.

cotranscription of the adjacent *vanH*, *vanA*, *vanX*, *vanY* and *vanZ* in response to the presence of vancomycin (31,76,79). VanH and VanA are implicated in the synthesis of modified peptidoglycan precursors (see below). VanY is postulated to contribute to glycopeptide resistance by depleting the intracellular pool of D-alanyl-D-alanine (vancomycin target). Finally, the roles of VanX and VanZ have not yet been elucidated.

An intragenic fragment of *vanA* hybridized to DNA from enterococci exhibiting high-level resistance to vancomycin and teicoplanin (the typical VanA phenotype), but not from those exhibiting low-level resistance to vancomycin only or enterococci susceptible to glycopeptides (17,58). In addition, gram-positive organisms intrinsically resistant to vancomycin, such as *Leuconostoc* spp., *Lactobacillus* spp., *Pediococcus* spp., *Erysipelothrix rhusiopathiae*, actinomycetes that produce the glycopeptides, and staphylococci resistant to teicoplanin failed to hybridize with the *vanA* probe (17). The origin of glycopeptide resistance, therefore, remains unclear.

Enterococci containing *vanB* have variable levels of resistance to vancomycin (MICs, 8-1024  $\mu\text{g/ml}$ ) but usually remain susceptible to teicoplanin (MICs,  $\leq 1$   $\mu\text{g/ml}$ ) (26,58,70,78). This resistance has been associated with the production of a novel 39.5 kDa membrane-associated protein termed VanB (2,78). In certain strains, it is transferable by conjugation to other enterococci although no plasmid could be demonstrated (58). Vancomycin but not teicoplanin induces the synthesis of VanB which explains why most of these strains test susceptible to teicoplanin (2,78). However, vancomycin-induced VanB strains or constitutive VanB-producer mutants, which can arise from a single step mutation, test resistant to teicoplanin (30). In other words, once VanB is produced, it confers resistance to teicoplanin although this glycopeptide does not itself cause induction. Teicoplanin-resistant mutants have been recovered from enterococci of the VanB phenotype both *in vitro* after selection on teicoplanin-containing agar and *in vivo* from a patient infected with an *E. faecium* strain of the VanB phenotype who received therapy with vancomycin intermittently (35,70). Interestingly, vancomycin-dependent mutants of *vanB*-containing *E. faecalis* and *E. faecium* have been recently reported (23,28). It is suggested that the production of the normal D-alanine:D-alanine ligase of these isolates is decreased, and thus they can only grow when vancomycin is present in the culture medium since its presence induces the production of the abnormal D-

alanine:D-alanine ligase encoded by *vanB* (23,28).

Enterococci containing *vanC* have low-level resistance to vancomycin (MICs, 8-32  $\mu\text{g/ml}$ ) and are susceptible to teicoplanin (MIC,  $\leq 1$   $\mu\text{g/ml}$ ). This resistance phenotype appears to be constitutively expressed, is non transferable and its determinants appear to be normal cell components that are encoded on the chromosome (46,74,75). VanC resistance was originally recognized as a property of motile enterococci, *E. gallinarum* and *E. casseliflavus*. The gene conferring low-level resistance to vancomycin was cloned and sequenced from a strain of *E. gallinarum* and termed *vanC* (18). A probe constructed from *vanC* hybridized to DNA from all strains of *E. gallinarum* examined, but it failed to hybridize to DNA from other species of enterococci resistant to low levels of vancomycin and susceptible to teicoplanin (46). Thus, *vanC* seems to be species-specific for *E. gallinarum*, and vancomycin resistance in *E. casseliflavus* is now classified as "VanC-like".

### Mechanisms of Glycopeptide Resistance

The glycopeptides, vancomycin and teicoplanin, bind irreversibly to the dipeptide D-alanyl-D-alanine located at the C terminus of the pentapeptide portion of the peptidoglycan precursor and thereby inhibit late stages in bacterial cell wall peptidoglycan synthesis (59). Normally, the C-terminal D-alanyl-D-alanine dipeptide is synthesized by a chromosomally encoded D-alanine:D-alanine ligase and then added to the peptidoglycan precursor-tripeptide. VanA has D-alanine:D-alanine ligase activity but preferentially catalyzes ester bond formation between D-alanine and various D-2-hydroxyacids, particularly D-lactate, resulting in the synthesis of the depsipeptide D-alanyl-D-lactate which is then added to the peptidoglycan precursor rather than the dipeptide D-alanyl-D-alanine (8). VanH, encoded by *vanH*, is a dehydrogenase that produces D-2-hydroxy acid substrates for VanA and is closely related to the D-lactate dehydrogenases found in *Leuconostoc* and *Lactobacillus*, which are gram-positive organisms intrinsically resistant to vancomycin (9). The depsipeptide has >1,000-fold reduced affinity for vancomycin compared to the normal D-alanyl-D-alanine dipeptide residue of susceptible enterococci; thus, peptidoglycan synthesis can continue despite the presence of vancomycin since the depsipeptide-terminating peptidoglycan precursor does not bind vancomycin (2,8,9,76).

The product of *vanY* is novel D,D-carboxypeptidase detected in enterococcal membrane fractions which hydrolyzes *in vitro* dipeptide D-alanyl-D-alanine, the depsipeptide D-alanyl-D-lactate and the C-terminal D-alanyl-D-alanine dipeptide of the normal pentapeptide precursor (2,4,30,31,80). This enzyme could contribute to glycopeptide resistance by depleting the intracellular pool of D-alanyl-D-alanine (2,30). VanY activity, however, is not essential for glycopeptide resistance (4).

The basis for vancomycin resistance in *vanB*- and *vanC*-containing enterococci are not yet as well characterized as in *vanA*-containing enterococci. Internal fragments of *vanB* derived from *E. faecalis* strains have been sequenced and the deduced amino acid sequence revealed 77% identity with the sequence of VanA (20,26). The structural relatedness of VanB and VanA suggests that the *vanB* product could also be involved in the synthesis of modified peptidoglycan precursors with decreased affinity for vancomycin (6,20). In addition, vancomycin induces the production of a D,D-carboxypeptidase in VanB strains similar to that found in VanA strains (30). VanC, the translated product of *vanC* of *E. gallinarum*, shares 29 to 38% amino acid identity with VanA and with D-alanine:D-alanine ligases of *E. coli*, respectively (18), and 37% amino acid identity with VanB (20,26). However, VanC does not seem to synthesize D-alanyl-D-lactate (18).

### Problems Caused by Vancomycin Resistance and Therapeutic Options

The treatment of vancomycin-resistant enterococci is a major clinical problem. Therapy for severe enterococcal infections such as endocarditis have traditionally included a synergistic combination of a cell wall-active antibiotic and an aminoglycoside. Penicillin or ampicillin plus gentamicin or streptomycin administered for 4 to 6 weeks are time honored regimens for endocarditis due to enterococci "normally susceptible" to penicillin and to aminoglycosides. Vancomycin provides an alternative in cases of patients allergic to penicillin or infected with enterococci that produce B-lactamase or that are resistant to achievable concentrations of penicillins. Vancomycin resistance eliminates the synergistic activity usually achieved by vancomycin-aminoglycoside combinations, thus leaving the B-lactams as the only choice to combine with the aminoglycoside. However, many of the vancomycin-resistant enterococci recently reported have shown multiple resistance, including high-level resistance to

ampicillin (MIC,  $\geq 200$   $\mu\text{g/ml}$ ) and to aminoglycosides (14,32,61). The optimal antibiotic therapy for these newly emerged multidrug-resistant enterococci is currently unknown.

Exposure of some strains of *E. faecium* moderately or highly resistant to vancomycin and moderately resistant to penicillin to low concentrations of vancomycin (10  $\mu\text{g/ml}$ ) causes the organisms to become more susceptible to B-lactams; that is, there is a decrease in the MICs of penicillin or ampicillin into a clinical useful range when vancomycin resistance is expressed (1,10,11,64). In such cases, combination of penicillin and vancomycin results in an inhibitory effect against isolates resistant to both B-lactams and glycopeptides (24,43,64,81). This combination, however, is not bactericidal as tested by time-kill experiments and the addition of an aminoglycoside is still necessary to achieve a bactericidal effect (11,24). In animal models, the combination of penicillin-vancomycin-gentamicin has been successful in the treatment endocarditis due to vancomycin-resistant *E. faecium* either moderately or highly resistant to penicillin without high-level resistance to gentamicin (10,11). While this triple drug combination can be active for some penicillin and vancomycin resistant strains that lack high-level resistance to aminoglycosides. Caron et al (11) recently reported the emergence of a residual subpopulation of bacteria resistant to the synergistic effect of penicillin-vancomycin combination after a penicillin- and vancomycin-resistant strain of *E. faecium* was exposed to the combination of penicillin, vancomycin, and gentamicin both *in vitro* and *in vivo*. In addition, other investigators have not found efficacy of penicillin-vancomycin combination against *E. faecium* strains of the VanA phenotype whose MICs of ampicillin were  $\geq 128$   $\mu\text{g/ml}$  (14,24,32). The level of resistance to penicillin may be responsible for some of the differences in response to penicillin-vancomycin combination.

Although ciprofloxacin shows only marginal *in vitro* activity against enterococci when used alone (22,62), ampicillin plus ciprofloxacin has been reported to show bactericidal activity against *E. faecium* highly resistant to penicillin and vancomycin if the MIC of ciprofloxacin was  $\leq 8$   $\mu\text{g/ml}$  (25,42). Nevertheless, there seems to be a species-to-species difference since combinations of ciprofloxacin and B-lactams have been indifferent against strains of *E. faecalis* and *E. avium* (22,52,65). Combination of either ciprofloxacin or ofloxacin with novobiocin, a DNA gyrase-inhibitor, demonstrated bactericidal

activity *in vitro* against ampicillin- and vancomycin-resistant strains of *E. faecium* but only for quinolone- and novobiocin-susceptible strains (25,42). Novobiocin alone demonstrated only inhibitory activity against strains of *E. faecium* at concentrations within the ranges achievable in serum, but it was less active against strains of *E. faecalis* (16,25,42). Therapeutics limitations to the use of novobiocin are toxicity, high serum protein binding, and potential emergence of resistance.

Rifampin shows *in vitro* activity against many enterococci, but it is generally bacteriostatic against these organisms (25,50,68). Treatment of experimental pyelonephritis and endocarditis due to *E. faecalis* demonstrated no benefit when rifampin was added to a cell-wall active agents such as ampicillin or vancomycin (36,57). Indeed, rifampin was antagonistic to B-lactams and to novobiocin when studied *in vitro*, probably due to the bacteriostatic activity of rifampin against enterococci (25,50). However, Livornese et al (47) successfully treated two cases of bacteremia due to vancomycin-resistant *E. faecium* with the combination of rifampin, ciprofloxacin and gentamicin. These investigators showed *in vitro* that combinations of ciprofloxacin plus rifampin with or without gentamicin were bactericidal for the *E. faecium* strain studied.

Teicoplanin has demonstrated good efficacy for the treatment of severe infections due to teicoplanin-susceptible enterococci (15). Since *vanB* and *vanC*-containing enterococci remain susceptible to teicoplanin, teicoplanin alone or in combination with an aminoglycoside when a bactericidal effect is sought are therapeutic options for these organisms. In an animal model of endocarditis, high-dose teicoplanin alone and standard-dose teicoplanin or vancomycin in combination with gentamicin have been effective for the treatment of a strain of *E. faecium* resistant to low levels of vancomycin and susceptible to teicoplanin (21). A major limitation to the use of teicoplanin for infections due to *vanB*-containing enterococci is the potential development of constitutive resistance to this glycopeptide (see above) (35,70).

Nitrofurantoin is a drug active against *E. faecalis* (MIC, 25 $\mu$ g/ml) which is suitable for treatment of urinary tract infections due to susceptible enterococci (MIC,  $\leq$  32  $\mu$ g/ml). Resistance to chloramphenicol among *E. faecalis* strains is not uncommon. Tofte et al (68) reported an MIC-range of chloramphenicol from 0.39-25  $\mu$ g/ml (MIC<sub>90</sub>, 12.5  $\mu$ g/ml) among 152 urine isolates of *E. faecalis*. Tucker et al (69), recently reported that all *E. faecium*

isolates highly resistant to ampicillin, vancomycin and streptomycin recovered from 18 patients during an outbreak of infections were susceptible to chloramphenicol (MIC,  $\leq$  4  $\mu$ g/ml). Other investigators found that among 26 distinct clinical isolates of *E. faecium* highly resistant to gentamicin and vancomycin 16 and 10 strains were susceptible to chloramphenicol and to tetracycline (MIC,  $<$  2  $\mu$ g/ml), respectively (12). Minocycline has also showed *in vitro* activity against vancomycin resistant *E. faecium* (16). Therefore, nitrofurantoin, chloramphenicol and tetracyclines are potential drugs to investigate for *in vitro* activity against vancomycin-resistant enterococci.

An important problem for the clinical laboratory is the relatively difficult detection of some vancomycin-resistant enterococci by disk diffusion or automated methods, particularly those with low-level resistance (MICs of vancomycin, 8-64  $\mu$ g/ml) such as is seen in *vanB*- and *vanC*-containing enterococci, and *E. casseliflavus* isolates (61). That is, organisms resistant by MIC testing were classified as susceptible by disk diffusion or automated methods. To overcome this problem, the National Committee for Clinical Laboratory Standards (NCCLS) has recommended changes for vancomycin disk diffusion testing criteria (67). However, it is still recommended to determine the MIC of vancomycin for those enterococcal strains exhibiting intermediate zones in disk diffusion assays, particularly in cases of severe enterococcal infections (54). An additional problem was noted by Quintiliani et al (58), who reported three *vanB*-containing enterococcal isolates that were resistant to vancomycin by disk diffusion but susceptible by MIC determination.

Finally, glycopeptide resistance has been transferred *in vitro* to other susceptible gram-positive organisms such as *Streptococcus sanguis*, *Streptococcus pyogenes*, *Streptococcus lactis*, *Bacillus thuringiensis*, and *Listeria monocytogenes* (7,17,44,45,72). Transfer of high-level vancomycin resistance by conjugation has been recently obtained between *E. faecalis* and *Staphylococcus aureus* both *in vitro* and on the skin of mice; worrisome is the fact that the second is a condition similar to that present in nature (56). Fortunately, clinical strains of *S. aureus* resistant to vancomycin have not been reported so far (56).

Vancomycin resistant enterococci represent a challenge for the clinician and the clinical microbiologist because of the increased occurrence of these organisms particularly in hospitalized patients, the concomitant resistance of many isolates to other agents including

high-level resistance to aminoglycosides and B-lactams, the sometimes difficult detection of vancomycin resistance by routine susceptibility testing, and the lack of reliable antimicrobial agents available for clinical use. This situation obligates the clinical microbiology laboratory to try to identify the most favorable agent(s) among the few currently available antimicrobial agents active against multiresistant enterococci, and warnings the physician to use antibiotics appropriately and to comply with the infections control policies to try to prevent further spread of these resistant organisms.

### References

- Al-Obeid S, Billot-Klein D, van Heijenoort J, Collatz E, Gutmann P and Duval J. Replacement of the essential penicillin-binding-protein 5 by high-molecular mass PBSs may explain the vancomycin- $\beta$ -lactam synergy in low-level vancomycin-resistant *Enterococcus faecium* D366. *FEMS Microbiol Lett* 1992;91:79-84.
- Al-Obeid S, Collatz E, Gutmann L. Mechanism of resistance to vancomycin in *Enterococcus faecium* D366 and *Enterococcus faecalis* A256. *Antimicrob Agents Chemother* 1990;34:252-6.
- Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob Agents Chemother* 1993;37:1563-71.
- Arthur M, Molinas C, Courvalin P. Sequence of the *vanY* gene required for production of a vancomycin-inducible D, D-carboxypeptidase in *Enterococcus faecium* BM4147. *Gene* 1992;120:111-4.
- Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* 1993;175:117-27.
- Billot-Klein D, Gutmann L, Collatz E, van Heijenoort J. Analysis of peptidoglycan precursors in vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 1992;36:1487-90.
- Brisson-Noel A, Dutka-Malen S, Molinas C, Leclercq R, Courvalin P. Cloning and heterospecific expression of the resistance determinant *vanA* encoding high-level resistance to glycopeptides in *Enterococcus faecium* BM4147. *Antimicrob Agents Chemother* 1990;34:924-7.
- Bugg TDH, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Identification of vancomycin resistance protein VanA as a D-alanine:D-alanine ligase of altered substrate specificity. *Biochemistry* 1991;30:2017-21.
- Bugg TDH, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry* 1991;30:10408-15.
- Caron F, Carbon C, Gutmann L. Triple-combination penicillin-vancomycin-gentamicin for experimental endocarditis caused by a moderately penicillin- and highly glycopeptide-resistant isolate of *Enterococcus faecium*. *J Infect Dis* 1991;164:888-93.
- Caron F, Lemeland JF, Humbert G, Klare I, Gutmann L. Triple combination penicillin-vancomycin-gentamicin for experimental endocarditis by highly penicillin- and glycopeptide-resistant isolate of *Enterococcus faecium*. *J Infect Dis* 1993;168:681-6.
- Casewell MW, Seyed-Akhavani M, Wade J. *In-vitro* activity of RP 59500 against vancomycin-resistant *Enterococcus faecium* also resistant to >512 mg/L of gentamicin. Abstr 1058, 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New Orleans, LA., 1993.
- CDC: Nosocomial enterococci resistant to vancomycin--United States 1989-1993. *MMWR* 1993;42:597-9.
- Cercenado E, Eliopoulos GM, Wennersten CB, Moellering RC Jr. Absence of synergistic activity between ampicillin and vancomycin against highly vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 1992;36:2201-3.
- Chenoweth C, Schaberg D. The epidemiology of enterococci. *Eur J Clin Microbiol Infect Dis* 1990;9:80-9.
- Cooper B, Robinson A, Freeman C, Quintiliani R, Mazens-Sullivan M, Nightingale C, Quintiliani R. Antimicrobial susceptibility of vancomycin resistant enterococci. Abstr 1053, 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New Orleans, LA, 1993.
- Dutka-Malen S, Leclercq R, Coutant V, Duval J, Courvalin P. Phenotypic and genotypic heterogeneity of glycopeptide resistance determinants in gram-positive bacteria. *Antimicrob Agents Chemother* 1990;34:1875-9.
- Dutka-Malen S, Molinas C, Arthur M, Courvalin P. Sequence of the *vanC* gene of *Enterococcus gallinarum* BM4174 encoding a D-alanine:D-alanine ligase-related protein necessary for vancomycin resistance. *Gene* 1992;112:53-8.
- Eliopoulos GM, Eliopoulos CT. Therapy of enterococcal infections. *Eur J Clin Microbiol Infect Dis* 1990;9:118-26.
- Evers S, Sahm DF, Courvalin P. The *vanB* gene of vancomycin-resistant *Enterococcus faecalis* V583 is structurally related to genes encoding D-Ala:D-Ala ligases and glycopeptide-resistance proteins VanA and VanC. *Gene* 1993;124:143-4.
- Fantin B, Leclercq R, Arthur M, Duval J, Carbon C. Influence of low-level resistance to vancomycin on efficacy of teicoplanin and vancomycin for treatment of experimental endocarditis due to *Enterococcus faecium*. *Antimicrob Agents Chemother* 1991;35:1570-5.
- Fernandez-Guerrero M, Rouse MS, Henry NK, Geraci JE, Wilson WR. *In vitro* and *in vivo* activity of ciprofloxacin against enterococci isolated from patients with infective endocarditis. *Antimicrob Agents Chemother* 1987;31:430-3.
- Fraimow H, Venuti E, Dean J. Mechanism of vancomycin dependence of a vancomycin requiring clinical *Enterococcus faecalis* isolate. Abstr 117, 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New Orleans, LA., 1993.
- Fraimow HS, Venuti E. Inconsistent bactericidal activity of triple-combination therapy with vancomycin, ampicillin, and gentamicin against vancomycin-resistant, highly ampicillin-resistant *Enterococcus faecium*. *Antimicrob Agents Chemother* 1992;36:1563-6.
- French P, Venuti E, Fraimow HS. *In vitro* activity of novobiocin against multiresistant strains of *Enterococcus faecium*. *Antimicrob Agents Chemother* 1993;37:2736-9.
- Gold HS, Ünal S, Cercenado E, Thauvin-Eliopoulos C, Eliopoulos GM, Wennersten CB, Moellering RC Jr. A gene conferring resistance to vancomycin but not teicoplanin in isolates of *Enterococcus faecalis* and *Enterococcus faecium* demonstrates homology with *vanB*, *vanA* and *vanC* genes of enterococci. *Antimicrob Agents Chemother* 1993;37:1604-9.
- Green M, Barbadora K, Michaels M. Recovery of vancomycin-

- resistant gram-positive cocci from pediatric liver transplant recipients. *J Clin Microbiol* 1991;29:2503-6.
28. Green M, Schlaes JH, Barbadora K, Schlaes DM. Vancomycin-dependent *Enterococcus faecium*: a preliminary characterization. Abstr 118, 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New Orleans, LA., 1993.
  29. Guiot HFL, Peetermans WE, Sebens FW. Isolation of vancomycin-resistant enterococci in haematologic patients. *Eur J Clin Microbiol Infect Dis* 1991;10:32-4.
  30. Gutmann L, Billot-Klein D, Al-Obeid S, Klare I, Francoual S, Collatz E, van Heijenoort J. Inducible carboxypeptidase activity in vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 1992;36:77-80.
  31. Handwerger S. Alterations in peptidoglycan precursors and vancomycin susceptibility in Tn917 insertion mutants of *Enterococcus faecalis* 221. *Antimicrob Agents Chemother* 1994;38:473-5.
  32. Handwerger S, Perlman DC, Altarac D, McAuliffe V. Concomitant high-level vancomycin and penicillin resistance in clinical isolates of enterococci. *Clin Infect Dis* 1992;14:655-61.
  33. Handwerger S, Raucher B, Altarac D, Monka J, Marchione S, Singh KV, Murray BE, Wolff J, Walters B. Nosocomial outbreak due to *Enterococcus faecium* highly resistant to vancomycin, penicillin and gentamicin. *Clin Infect Dis* 1993;16:750-3.
  34. Handwerger S, Tomasz A. Antibiotic tolerance among clinical isolates of bacteria. *Rev Infect Dis* 1985;7:368-86.
  35. Hayden MK, Trenholme GM, Schultz JE, Sahn DF. *In vivo* development of teicoplanin resistance in a VanB *Enterococcus faecium* isolate. *J Infect Dis* 1993;167:1224-7.
  36. Ingerman M, Pitsakis PG, Rosenberg A, Hessen MT, Abrutyn E, Murray BE, Levison ME.  $\beta$ -lactamase production in experimental endocarditis due to aminoglycoside-resistant *Streptococcus faecalis*. *J Infect Dis* 1987;155:1226-32.
  37. Johnson AP, Uttley AH, Woodford N, George RC. Resistance to vancomycin and teicoplanin: an emerging clinical problem. *Clin Microbiol Rev* 1990;3:280-91.
  38. Jones RN, Erwin ME, and the Enterococcus Study Group. Emerging multiply resistant enterococci (MRE) among clinical isolates: prevalence data from 97 medical centers. Abstr 1052, 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New Orleans, LA., 1993.
  39. Kaplan AH, Gilligan PH, Facklam RR. Recovery of resistant enterococci during vancomycin prophylaxis. *J Clin Microbiol* 1988;26:1216-8.
  40. Karanfil LV, Murphy M, Josephson A, Gaynes R, Mandel L, Hill BC, Swenson JM. A cluster of vancomycin-resistant *Enterococcus faecium* in an intensive care unit. *Infect Control Hosp Epidemiol* 1992;13:195-200.
  41. Kim MJ, Weiser M, Gottschall S, Randall EL. Identification of *Streptococcus faecalis* and *Streptococcus faecium* and susceptibility studies with newly developed antimicrobial agents. *J Clin Microbiol* 1987;25:787-90.
  42. Landman D, Mobaraki NK, Quale JM. Novel antibiotic regimens against *Enterococcus faecium* resistant to ampicillin, vancomycin, and gentamicin. *Antimicrob Agents Chemother* 1993;37:1904-8.
  43. Leclercq R, Bingen E, Su QH, Lambert-Zechovski N, Courvalin P, Duval J. Effects of combinations of  $\beta$ -lactams, daptomycin, gentamicin, and glycopeptides against glycopeptide-resistant enterococci. *Antimicrob Agents Chemother* 1991;35:92-8.
  44. Leclercq R, Deriot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Engl J Med* 1988;319:157-61.
  45. Leclercq R, Deriot E, Weber M, Duval J, Courvalin P. Transferable vancomycin and teicoplanin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* 1989;33:10-5.
  46. Leclercq R, Dutka-Malen S, Duval J, Courvalin P. Vancomycin resistance gene *vanC* is specific to *Enterococcus gallinarum*. *Antimicrob Agents Chemother* 1992;36:2005-8.
  47. Livornese LL Jr, Dias S, Samel C, et al. Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic thermometers. *Ann Intern Med* 1992;117:112-6.
  48. Longfield RN, Wortham WG, Fletcher LL, Nauscheutz WE. Clustered bacteremias in a hemodialysis unit: cross-contamination of blood tubing from ultrafiltrate waste. *Infect Control Hosp Epidemiol* 1992;13:160-4.
  49. Luticken R, Kunstmann G. Vancomycin-resistant *Streptococcaceae* from clinical material. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg 1 Orig Reihe A* 1988;267:379-82.
  50. Moellering RC Jr, Wennersten CB. Therapeutic potential of rifampin in enterococcal infections. *Rev Infect Dis* 1983;5(Suppl): 528-32.
  51. Montecalvo MA, Gedris C, Issah A, Carbonaro C, et al. An outbreak of vancomycin resistant *Enterococcus faecium* (VREF) and an adult oncology unit. Abstr 1169, 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Anaheim, Ca., 1992.
  52. Moody JA, Peterson LR, Gerding DN. *In vitro* activity of ciprofloxacin combined with azlocillin. *Antimicrob Agents Chemother* 1985;28:849-50.
  53. Murray BE. The life and times of the enterococcus. *Clin Microbiol Rev* 1990;3:46-65.
  54. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing, 3rd informational supplement. Publication M100-S3, National Committee for Clinical Laboratory Standards Villanova, Pa., 1991.
  55. Nicas TI, Wu CYE, Hobbs JN Jr, Preston DA, Allen NE. Characterization of vancomycin resistance in *Enterococcus faecium* and *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1989;33:1121-4.
  56. Noble WD, Virani Z, Cree RCA. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett* 1992;93: 195-8.
  57. Oill PA, Kalmanson GM, Guze LB. Rifampin, ampicillin, streptomycin, and their combinations in the treatment of enterococcal pyelonephritis in rats. *Antimicrob Agents Chemother* 1981;20:491-2.
  58. Quintiliani R Jr, Evers S, Courvalin P. The *vanB* gene confers various levels of self-transferable resistance to vancomycin in enterococci. *J Infect Dis* 1993;167:1220-3.
  59. Reynold PE. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur J Clin Microbiol Infect Dis* 1989; 8:943-50.
  60. Rubin LG, Tucci V, Cercenado E, Eliopoulos G, Isenberg HD. Vancomycin-resistant *Enterococcus faecium* in hospitalized children. *Infect Control Hosp Epidemiol* 1992;13:700-5.
  61. Sahn DF, Kissinger J, Gilmore JS, Murray PR, Mulder R, Solliday J, Clarke B. *In vitro* susceptibility studies of vancomycin-resistant *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1989;33: 1588-91.
  62. Sahn DF, Koburov GT. *In vitro* activities of quinolones against

- enterococci resistant to penicillin-aminoglycoside synergy. *Antimicrob Agents Chemother* 1989;33:71-7.
63. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. *Am J Med* 1991; 91 (Suppl 3B):72S-75S.
64. Shlaes DM, Etter L, Gutmann L. Synergistic killing of vancomycin-resistant enterococci of classes A, B, and C by combinations of vancomycin, penicillin, and gentamicin. *Antimicrob Agents Chemother* 1991;35:776-9.
65. Smith SM, Eng RHK. Interaction of ciprofloxacin with ampicillin and vancomycin for *Streptococcus faecalis*. *Diagn Microbiol Infect Dis* 1988;9:239-43.
66. Stevenson KB, Murray EW, Sarubbi FA. Enterococcal meningitis: report of four cases and review. *Clin Infect Dis* 1994;18:233-9.
67. Swenson JM, Ferraro MJ, Sahn DF, Charache P, The National Committee for Clinical Laboratory Standards Working Group on Enterococci, Tenover FC. New vancomycin disk diffusion breakpoints for enterococci. *J Clin Microbiol* 1992;30:2525-8.
68. Tofte RW, Solliday J, Crossley KB. Susceptibilities of enterococci to twelve antibiotics. *Antimicrob Agents Chemother* 1984;25:532-3.
69. Tucker L, Peterson L, Noskin G, Cooper I, Reisberg B. Activity of 32 antimicrobial agents alone and in combination against high level vancomycin resistant *Enterococcus faecium*. Abstr 1061, 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New Orleans, LA., 1993.
70. Unal S, Cercenado E, Eliopoulos G, Moellering RC Jr. A novel type of transferable glycopeptide resistance among enterococci isolated in the United States. Abstr 1484, 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Anaheim, Ca., 1992.
71. Uttley AHC, Collins CH, Naidoo J, George RC. Vancomycin-resistant enterococci. *Lancet* 1988;i:57-8.
72. Uttley AHC, George RC, Naidoo J, Woodford N, et al. High-level vancomycin-resistant enterococci causing hospital infections. *Epidem Inf* 1989;103:173-81.
73. Venditti M, Biavasca F, Varaldo PE, Macchiarelli A, De Biase L, Marino B, Serra P. Catheter-related endocarditis due to glycopeptide-resistant *Enterococcus faecalis* in a transplanted heart. *Clin Infect Dis* 1993;17:524-5.
74. Vincent S, Knight RG, Green M, Sahn DF, Shlaes DM. Vancomycin susceptibility and identification of motile enterococci. *J Clin Microbiol* 1991;29:2335-7.
75. Vincent S, Minkler P, Binczewski B, Etter L, Shlaes DM. Vancomycin resistance in *Enterococcus gallinarum*. *Antimicrob Agents Chemother* 1992;36:1392-9.
76. Walsh CT. Vancomycin resistance: decoding the molecular logic. *Science* 1993;261:308-9.
77. Watanakunakorn C, Bakie C. Synergism of vancomycin-gentamicin and vancomycin-streptomycin against enterococci. *Antimicrob Agents Chemother* 1973;4:120-4.
78. Williamson R, Al-Obeid S, Shlaes JH, Goldstein FW, Shlaes DM. Inducible resistance to vancomycin in *Enterococcus faecium* D366. *J Infect Dis* 1989;159:1095-104.
79. Wright GD, Holman TR, Walsh CT. Purification and characterization of VanR and the cytosolic domain of VanS: a two-component regulatory system required for vancomycin resistance in *Enterococcus faecium* BM4147. *Biochemistry* 1993;32:5057-63.
80. Wright GD, Molinas C, Arthur M, Courvalin P, Walsh CT. Characterization of VanY, a DD-carboxypeptidase from vancomycin-resistant *Enterococcus faecium* BM4147. *Antimicrob Agents Chemother* 1992;36:1514-8.
81. Zigelboim-Daum S, Moellering RC Jr. Mechanisms and significance of antimicrobial resistance in enterococci. In: Actor P, Deneo-Moore L, Higgins ML, Satton MRJ, Shockman GD, eds. Antibiotic inhibition of bacterial cell surface assembly and function. Washington, DC: American Society for Microbiology, 1988:603-15.