

## Antimicrobial Resistance in Enterococci

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*Enterococci have emerged as a major nosocomial pathogen and as an ever-increasing problem in antimicrobial resistance. They are ubiquitous in the intestinal flora of humans and animals and inherently resistant to a wide array of antimicrobial agents, and, more alarmingly, they seem to have a potential facility for acquiring new resistance determinants, including  $\beta$ -lactamase production, high-level resistance to aminoglycosides, and recently, glycopeptide resistance. Collectively, all of these properties make enterococci one of most difficult nosocomial pathogens to treat and control today. The purpose of this review was to examine the epidemiology, the mechanisms, and laboratory detection of resistance of enterococci to the two major groups of antibiotics: aminoglycosides and glycopeptides.*

**Key Words:** Enterococci, high-level resistance to aminoglycosides, vancomycin, teicoplanin, glycopeptide resistance

Enterococci are normal inhabitants of the intestinal tract of humans and animals, as well as colonizers of the human oral cavity, vagina and hepatobiliary tract. Among more than a dozen species of enterococci currently recognized, two species, *Enterococcus faecalis* and *E. faecium*, have been isolated most frequently from clinical cases, accounting for 80-90% and 5-10%, respectively (Facklam and Sahm, 1995). In recent years, however, *E. faecium* has become more common, probably because of its greater antibiotic resistance (Jones *et al.* 1995; Moellering, 1998). *E. avium*, *E. durans*, *E. gallinarum*, *E. casseliflavus*, *E. hirae*, and *E. raffinosus* are the other species of enterococci that have been reported from clinical sources, but still selectively account for few clinical isolates.

Despite being considered to have relatively low virulence compared to other gram-positive or-

ganisms enterococci in recent years have emerged as the nosocomial pathogens of the 1990s (Moellering, 1992; Spera and Farber, 1992; Emori and Gaynes, 1993; Centers for Disease Control and Prevention [CDC], 1997b). Several factors have contributed to the emergence of enterococci as important pathogens, including their ubiquitous distribution as intestinal flora and the widespread use of broad-spectrum antibiotics and invasive catheters (Chenoweth and Schaberg, 1996), but perhaps most important is their extensive resistance to a wide array of antimicrobial agents. It is these properties that allow the organism to survive and multiply with a selective advantage over other fecal flora in a hospital environment where antimicrobial agents are heavily used.

### ANTIMICROBIAL RESISTANCE IN ENTEROCOCCI

Antimicrobial resistance can be divided into two general types, intrinsic (or inherent) and acquired (Table 1). All enterococci are intrinsically resistant to a number of antimicrobial agents. They exhibit

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Table 1. Antimicrobial resistance in enterococci

Resistance	Characteristics	Genes
<b>Intrinsic</b>		
Penicillins	Low-affinity PBPs, relative resistance, tolerance	Chromosomal
Cephalosporins	Diminished affinity for PBPs 4, 5, 6	Chromosomal
Clindamycin	Low level	Chromosomal
Aminoglycosides	Low level due to permeability/low uptake	Chromosomal
Trimethoprim/ Sulfamethoxazole	Resistance in vivo due to ability of organism to use exogenous folates	Chromosomal
Quinolones	Permeability/reduced uptake	Chromosomal
Glycopeptides	Low level in <i>E. casseliflavus</i> and <i>E. gallinarum</i>	Chromosomal ( <i>vanC</i> )
<b>Acquired</b>		
Penicillins	Increased resistance due to altered PBPs; High-level resistance due to $\beta$ -lactamase	Chromosomal Transposon/plasmid
Aminoglycosides	High level due to production of AMEs	Transposon/plasmid
Macrolides/ Lincosamides	Reduced ribosomal binding due to enzymes that methylate 23S rRNA	Transposon/plasmid
Tetracycline	Partly due to increased influx of drug from cell	Transposon/plasmid
Chloramphenicol	Chloramphenicol acetyltransferase	Plasmid
Quinolones	High level due to gyrase mutation	Chomosomal
Glycopeptides	High level due to altered ligase	Transposon/plasmid ( <i>vanA</i> and <i>vanB</i> )

Adapted from (Chenoweth and Schaberg, 1996; French, 1998).

AMEs: aminoglycoside-modifying enzymes; PBPs: penicillin-binding proteins.

low levels of resistance to  $\beta$ -lactam agents, as evidenced by the selectively-high MICs of most penicillins, including penicillin G and ampicillin. The MICs of penicillin are 2–8  $\mu\text{g}/\text{mL}$  for *E. faecalis* and 16–32  $\mu\text{g}/\text{mL}$  for *E. faecium*, compared to 0.02–0.10  $\mu\text{g}/\text{mL}$  for *Streptococcus bovis* (Moellering and Krogstad, 1979). Enterococci also exhibit "tolerance" to all agents that inhibit cell-wall synthesis, including  $\beta$ -lactams and vancomycin. For example, the MBCs of penicillin are about the same as MIC for *Streptococcus bovis*, whereas a wide discrepancy (as much as 100-fold) exists between the MIC and the MBC of penicillin against enterococci (Moellering and Krogstad, 1979). This means that even in high concentrations, penicillin is bacteriostatic, not bactericidal, against enterococci. For this reason, enterococcal endocarditis is usually treated with the synergistic and bactericidal combination of penicillin/ampicillin plus an aminoglycoside. However, this therapy will not be effective for the enterococci that have acquired high-level aminoglycoside resistance (Murray, 1990).

All enterococci exhibit significant resistance to semisynthetic penicillins and cephalosporins, and these antibiotics, such as penicillin, are not bactericidal against enterococci. Enterococci also have inherent low-level resistance to clindamycin, aminoglycosides, quinolones, and co-trimoxazole. The inherent low-level vancomycin resistance in *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* will be discussed later.

In addition to this natural low-level resistance, enterococci readily acquire high-level resistance to other drugs. Examples of acquired resistance include resistance to penicillins by means of additional low-affinity PBPs or the production of  $\beta$ -lactamase, chloramphenicol, macrolides, tetracyclines, fluoroquinolones, high levels of aminoglycosides, and glycopeptides. Collectively, all of these resistant traits make enterococci one of the most difficult nosocomial pathogens to treat.

However, it is the high-level resistance to aminoglycosides and glycopeptides that poses the most serious problems for the treating physician and

infection-control personnel, and will be the subject of this review. Excellent reviews on the subject are also available elsewhere (Murray, 1990; Leclercq *et al.* 1992; Woodford *et al.* 1995; Leclercq and Courvalin, 1997; French, 1998; Moellering, 1998).

## HIGH-LEVEL RESISTANCE (HLR) TO AMINOGLYCOSIDES

The majority of infections caused by enterococci can be cured with bacteriostatic therapy when the patient has normal host defenses. Urinary tract infections or intraabdominal infections can be treated with penicillin or ampicillin alone as long as the infecting enterococcal strains are not highly resistant to the  $\beta$ -lactams. Likewise, vancomycin alone is effective as long as the organism is not resistant to this agent. However, for the successful treatment of enterococcal endocarditis and other serious enterococcal infections, the bactericidal combination of aminoglycosides and penicillin or ampicillin is required. Although enterococci are inherently resistant to low levels of aminoglycosides (MIC  $\geq 4$ – $\leq 250$   $\mu\text{g/mL}$ ), the addition of cell-wall synthesis inhibitors to aminoglycosides will result in enhanced killing by the synergistic action of the two antimicrobials.

When enterococci have acquired HLR to aminoglycosides as defined by MICs of  $\geq 2,000$   $\mu\text{g/mL}$ , however, the organism becomes resistant to the synergism, thus presenting a therapeutic problem for patients with serious enterococcal infections (Krogstad *et al.* 1978). The mortality due to bacteremia caused by enterococci with HLR to gentamicin was

higher than that due to bacteremia caused by enterococci without HLR to gentamicin (Noskin *et al.* 1991).

### Prevalence

Streptomycin was the aminoglycoside used clinically as part of combination therapy with penicillin or ampicillin for serious enterococcal infection until the early 1970s (Moellering *et al.* 1970), when the majority of enterococci was found to be resistant to high levels of this drug (MIC  $> 2,000$   $\mu\text{g/mL}$ ). However, enterococci with high-level resistance to gentamicin were also reported soon after and by the late 1980's its prevalence had reached up to 50% or higher in some areas (Medershi-Samoraj and Murray, 1983; Zervos *et al.* 1987; Grayson *et al.* 1991). Recent reports from Korea have shown that the prevalence of enterococci with high-level resistance to gentamicin, kanamycin (a surrogate marker for amikacin), and streptomycin is 60–65%, 64–73%, and 40–57%, respectively (Kim *et al.* 1992; Chong, 1993; Shin and Ryan, 1993). Concern over antibiotic resistance in enterococci increased further when many vancomycin-resistant isolates in the United States also proved to be resistant to  $\beta$ -lactams and aminoglycosides, leaving few therapeutic options.

### Biochemical mechanisms of resistance

Enterococci are resistant to aminoglycosides by three distinct mechanisms: 1) low permeability, 2) alteration of the target site, and 3) enzymatic modification of the antibiotic.

Uptake of aminoglycosides by bacterial cells requires oxidatively generated energy (Bryan and

**Table 2. Aminoglycoside-inactivating enzymes in enterococci**

High-level resistance	Inactivating enzyme	Cross resistant to other aminoglycosides
Streptomycin	6-nucleotidyltransferase	None
Kanamycin	3'-phosphotransferase	Amikacin
	4'-nucleotidyltransferase	Amikacin
Gentamicin	2"-phosphotransferase	Tobramycin, amikacin, and all other aminoglycosides except streptomycin
	6'-acetyltransferase	

Adapted from (Leclercq *et al.* 1992).

Van der Elzen, 1977). Enterococci, which have an anaerobic metabolism with a defect in oxidatively energizing the cell membrane, are intrinsically resistant to low levels of aminoglycosides caused by inefficient active transport of the antibiotic. High-level resistance to streptomycin has shown to be due to a single amino acid change in a 30S ribosomal protein, the target site of the antibiotic. This change eliminates the ability of streptomycin to bind to the ribosomal subunit and exerts its inhibitory effect on protein synthesis (Eliopoulos *et al.* 1984).

The most common mechanism for aminoglycoside resistance involves the conjugation of the antibiotic with one of three groups, an acetyl group, an adenylyl group, or a phosphoryl group, thereby rendering the aminoglycosides inactive (Krogstad *et al.* 1978). This high-level resistance is mediated by aminoglycoside-modifying enzymes (Table 2). With the pre-existing inherent deficiency for the uptake of aminoglycosides, the consequences of the action of the modifying enzymes are magnified toward extremely high MICs, e.g.,  $>2,000 \mu\text{g/mL}$ .

#### Screening for high-level resistance to aminoglycosides

In the case of severe enterococcal infections, it is necessary to screen for high-level resistance to aminoglycosides to determine the synergistic activity with a cell-wall-active antimicrobial agent. Only streptomycin, kanamycin, and gentamicin need to be studied. Recently, the National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antimicrobial Susceptibility Testing evaluated three of the most commonly used methods

(agar dilution, broth microdilution, and disk diffusion) to determine reliable conditions and methods for detection of high-level resistance to aminoglycosides in enterococci (Swenson *et al.* 1995). High-level aminoglycoside resistant mutants can be best screened by the agar dilution, broth microdilution, or disk diffusion methods under the conditions specified in Table 3.

In the case of HLR to streptomycin, streptomycin should not be used in combination with a  $\beta$ -lactam agent. In the case of HLR to kanamycin, kanamycin and amikacin should not be used in combination with a  $\beta$ -lactam agent. In the case of HLR to gentamicin, gentamicin, netilmicin, kanamycin, amikacin, tobramycin, or any other aminoglycosides except streptomycin should not be used in combination with  $\beta$ -lactam agents.

## GLYCOPEPTIDE RESISTANCE

### Epidemiology of vancomycin-resistant enterococci

With the increasing incidence of HLR to aminoglycosides and penicillins in the late 1980s, vancomycin had become the only choice of antibiotic available for the treatment of serious infections due to enterococci. Then in 1988, vancomycin-resistant *E. faecium* and *E. faecalis* were first described in Britain (Uttley *et al.* 1988) and this was followed soon afterward by outbreaks of VRE in hospitals in other European countries and the United States (Leclercq *et al.* 1988; Bingen *et al.* 1991; Frieden *et al.* 1993). Since these early reports, increasing numbers of outbreaks of VRE have been reported

Table 3. Screening tests for high-level resistance to aminoglycosides among enterococci

Method	Medium	Inoculum	Incubation time (h)	Concentration ( $\mu\text{g/mL}$ or $\mu\text{g/disk}$ )		
				Gentamicin	Kanamycin	Streptomycin
Agar dilution	BHI	$1 \times 10^6$ CFU/spot	24	500	500	2,000
Broth microdilution	BHI	$5 \times 10^6$ CFU/well	24	500	500	1,000
Disk diffusion	MHA	0.5 McFarland	18–24	120	120	300

Endpoint for each test: Agar dilution, any growth  $>1$  colony; broth microdilution, any growth; disk diffusion, 6 mm: HLR, 7–9 mm: inconclusive,  $\geq 10$  mm: susceptible (Swenson *et al.* 1995).

in North America and Europe (See Review by Leclercq and Courvalin, 1997; French, 1998). In U.S. hospitals, the percentage of nosocomial VRE increased from 0.3% in 1989 to 7.9% in 1993. Among patients in intensive care units, the increase was more dramatic; from 0.45% in 1989 to 13.6% in 1993, a 34-fold increase in a 4-year period (CDC, 1993). Since the first report of VRE in 1992 (Park *et al.* 1992) in Korea, fecal colonization by VRE has been reported to be 3.2%–9.5% in tertiary-care hospitals (Lee and Pai, 1997; Jeong *et al.* 1998), and the incidence of VRE in clinical specimens has been increased up to 7.5% at a university hospital (Lee *et al.* 1998).

The problem of VRE is two-fold. First, most nosocomial isolates of enterococci resistant to vancomycin are also resistant to other first-line antimicrobials for enterococcal infection, including penicillin and high levels of aminoglycosides (Moellering, 1992), thus limiting therapeutic options for patients infected with VRE. Secondly, and ever more seriously, there is concern that vancomycin resistance could be transferred from enterococci to a more virulent gram-positive pathogen such as staphylococci. Transfer has been shown to occur under laboratory conditions (Noble *et al.* 1992; Biavasco *et al.* 1996) and one of the vancomycin-resistant genes has been demonstrated in a clinical isolate of *Staphylococcus bovis* (Poyart *et al.* 1997). Clinical isolates of *S. aureus* with reduced suscep-

tibility to vancomycin have been reported in Japan (Hiramatsu *et al.* 1997) and the United States (CDC, 1997a), although vancomycin-resistant genes do not seem to be involved (Tenover *et al.* 1998).

### Properties of glycopeptide-resistant enterococci

Three phenotypes, VanA, VanB, and VanD, can be distinguished for acquired resistance to glycopeptides (Table 4). VanA strains are resistant to high levels of vancomycin and teicoplanin and the resistance, which is mediated by self-transferrable plasmids, is inducible by both vancomycin and teicoplanin. The VanB phenotype is characterized by inducible resistance to low to high levels of vancomycin, but remains susceptible to teicoplanin. Both VanA and VanB resistance are most commonly seen in *E. faecium* and *E. faecalis*. A VanD phenotype has been described recently in a single strain of *E. faecium*, which was shown to have moderate-level resistance to vancomycin and low-level resistance to teicoplanin (Perichon *et al.* 1997). The intrinsic low resistance to vancomycin with susceptibility to teicoplanin seen in *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* has been designated VanC (Leclercq *et al.* 1991; Navarro and Courvalin, 1994; Clark *et al.* 1998). Strains with VanC phenotype were rarely recovered from clinical specimens, but occasionally found as part of the normal fecal flora with little clinical significance

Table 4. Phenotypes of glycopeptide-resistant enterococci

Resistant type	Phenotype	MIC ( $\mu\text{g/mL}$ )		Transferable resistance	Species
		Vancomycin	Teicoplanin		
Acquired	VanA	64–1000	16–512	Yes	<i>E. faecium</i> <i>E. faecalis</i> <i>E. avium</i>
	VanB	4–1000	0.5–1	Yes	<i>E. faecalis</i> <i>E. faecium</i>
	VanD	16–64	2–4	NT	<i>E. faecium</i>
Intrinsic	VanC1 C2 C3	2–32	0.5–1	No	<i>E. gallinarum</i> <i>E. casseliflavus</i> <i>E. flavescens</i>

NT: not tested.

From Arthur and Courvalin, 1993; Navarro and Courvalin, 1994; Leclercq and Courvalin, 1997.

(Toye *et al.* 1997).

### Mechanism of resistance

Glycopeptides inhibit cell-wall synthesis by forming complexes with the peptidyl-D-alanyl-D-alanine termini of peptidoglycan precursors at the cell surface, which in turn block the formation of interpeptide bonds (Nieto and Perkins, 1971). In strains with glycopeptide resistance, the products of the resistant genes catalyze the formation of ester bonds between D-alanine and D-lactate to produce the depsipeptide D-Ala-D-Lac preferentially to the usual dipeptide D-Ala-D-Ala (Bugg *et al.* 1991). These modified precursors display a markedly-reduced affinity for vancomycin and teicoplanin and thus confer resistance to the inhibitory actions of the antibiotics, while cell wall synthesis continues to proceed because of a wider substrate specificity of the bacterial ligase.

On the basis of the mechanism described above, it would appear that production of the resistant gene *vanA* is sufficient to confer glycopeptide resistance; however, the mechanism of resistance is much more complicated. In fact, the production of D-Ala-D-Lac depends on the cooperative activity of three enzymes, VanA, VanH, and VanX, and the genes encoding three proteins are located on a plasmid and arranged within an operon (Leclercq and Courvalin, 1997; French, 1998).

### Screening for and detection of glycopeptide resistance

There are no doubts that VRE colonization and infection in hospital patients will continue to increase, and that the control of emergence and spread of these organisms will become more imperative. To prevent the spread of glycopeptide resistance, infection control measures have been recommended that involve decreasing the risk of colonization, interrupting transmission, and eliminating reservoirs (Hospital Infection Control Practices Advisory Committee, 1995). As well, it is crucial for laboratories to provide rapid and accurate susceptibility testing results for enterococci so that effective therapy and infection control measures can be initiated. For accurate detection, laboratories

should use reliable conventional broth and agar dilution methods rather than automated systems, which have been found to have difficulty in detecting low-level vancomycin resistance (Tenover *et al.* 1993; Tenover *et al.* 1995). In addition, a VRE screening plate consisting of brain-heart infusion agar containing 6 µg/mL of vancomycin has been found highly accurate in screening for vancomycin resistance (Swenson *et al.* 1994). Recently, PCR technique has also been used for the rapid detection and characterization of glycopeptide resistance (Dutka-Malen *et al.* 1995; Patel *et al.* 1997).

### CONCLUSION

The increasing resistance to glycopeptide and aminoglycosides in enterococci is causing a serious problem for clinicians, microbiologists, and infection-control personnel. For clinicians, there is the possibility that no effective antimicrobials would be available for serious enterococcal infections. For infection-control personnel, control of the emergence and spread of glycopeptide-resistant enterococci has become imperative because of the possibility that the resistance determinants may be transferred into other gram-positive organisms. Finally, for microbiologists it will be crucial to provide rapid and accurate susceptibility testing results so that effective therapy and infection-control measures for this important nosocomial pathogen can be initiated.

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