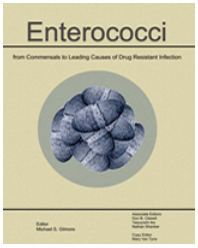
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Kristich CJ, Rice LB, Arias CA. Enterococcal Infection—Treatment and Antibiotic Resistance. 2014 Feb 6. In: Gilmore MS, Clewell DB, Ike Y, et al., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014-.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



Enterococcal Infection—Treatment and Antibiotic Resistance

Christopher J. Kristich, PhD,[1] Louis B. Rice, MD,[2] and Cesar A. Arias[3]

Created: February 6, 2014.

Introduction

The clinical importance of the genus *Enterococcus* is directly related to its antibiotic resistance, which contributes to the risk of colonization and infection. The species of the greatest clinical importance are *Enterococcus faecalis* and *Enterococcus faecium*. Although the resistance characteristics of these two species differ in important ways, they can generally be categorized as intrinsic resistance, acquired resistance, and tolerance.

Relative to the streptococci, enterococci are intrinsically resistant to many commonly used antimicrobial agents. All enterococci exhibit decreased susceptibility to penicillin and ampicillin, as well as high-level resistance to most cephalosporins and all semi-synthetic penicillins, as the result of expression of low-affinity penicillin-binding proteins. For many strains, their level of resistance to ampicillin does not preclude the clinical use of this agent. In fact, ampicillin remains the treatment of choice for enterococcal infections that lack other mechanisms for high-level resistance. Enterococci are also intrinsically resistant to clindamycin, which is mediated by the product of the *lsa* gene, although the mechanism remains poorly defined. Trimethoprim-sulfamethoxazole appears to be active against enterococci when tested *in vitro* on folate-deficient media, but fails in animal models, presumably because enterococci can absorb folate from the environment (Zervos & Schaberg, 1985). Enterococci also have a native resistance to clinically achievable concentrations of aminoglycosides, which precludes their use as single agents. Although *E. faecalis* is naturally resistant to quinupristin-dalfopristin, this combination is highly active against *E. faecium* strains that lack specific resistance determinants.

Enterococci are tolerant to the (normally) bactericidal activity of cell-wall active agents, such as β–lactam antibiotics and vancomycin. Tolerance implies that the bacteria can be inhibited by clinically achievable concentrations of the antibiotic, but will only be killed by concentrations far in excess of the inhibitory concentration. Enterococcal tolerance can be overcome by combining cell-wall active agents with an aminoglycoside. The mechanism by which β–lactam-aminoglycoside combinations yield synergistic bactericidal activity remains a mystery, but *in vitro* data indicate that a higher concentration of aminoglycoside enters cells that are also treated with agents that inhibit cell wall synthesis, which suggests that the cell wall active agents promote uptake of the aminoglycoside (Mohr, Friedrich, Yankelev, & Lamp, 2009).

Author Affiliations: 1 Department of Microbiology and Molecular Genetics, Center for Infectious Disease Research, Medical College of Wisconsin, Milwaukee, WI; Email: ckristich@mcw.edu. 2 Department of Medicine, Warren Alpert Medical School of Brown University, Providence, Rhode Island; Physician-in-Chief, Rhode Island and Miriam Hospitals; Email: LRICE@lifespan.org. 3 Department of Internal Medicine, Division of Infectious Diseases and Laboratory for Antimicrobial Research, University of Texas Medical School at Houston, Houston, TX, USA Molecular Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Bogotá, Colombia; Email: Cesar.Arias@uth.tmc.edu.

Corresponding author.

Tolerance is normally detected *in vitro* by plotting survival in kill curves, and can be observed for a number of antibiotic-bacteria combinations. I*n vitro* tolerance has an important impact on therapy for treating enterococcal infections. The treatment of endocarditis requires bactericidal therapy, due to the inaccessibility of the bacteria within the cardiac vegetations to the mammalian immune system. Recognition of synergism between penicillin-streptomycin led to an improvement in cure rates for enterococcal endocarditis, from approximately 40% to greater than 80% (Jensen, Frimodt-Møller, & Aarestrup, 1999; Rice & Carias, 1998). Despite considerable effort, investigators have yet to find other combinations of antibiotics that are synergistically bactericidal against enterococci.

In addition to intrinsic resistance and tolerance, enterococci have been extraordinarily successful at rapidly acquiring resistance to virtually any antimicrobial agent put into clinical use. Introduction of chloramphenicol, erythromycin and tetracyclines was quickly followed by the emergence of resistance, in some cases reaching a prevalence that precluded their empirical use. While the occurrence of ampicillin resistance in *E. faecalis* has been quite rare, there is now widespread, high-level resistance to ampicillin among clinical *E. faecium* isolates. High-level aminoglycoside resistance, which negates the synergism between cell-wall active agents and aminoglycosides, has been recognized for several decades. Vancomycin resistance is widely prevalent in *E. faecium*, although it remains relatively rare in *E. faecalis*. In response to the growing problem of vancomycin resistance in enterococci, the pharmaceutical industry has developed a number of newer agents that have activity against vancomycin-resistant enterococci (VRE). However, none of these newly licensed agents (quinupristin-dalfopristin, linezolid, daptomycin, tigecycline) has been entirely free of resistance. Thus, the widespread resistance of enterococci has had a substantial impact on our use of both empirical and definitive antibiotics for the treatment of enterococcal infections, a situation that is likely to persist for the foreseeable future.

Molecular Mechanisms of Antibiotic Resistance in Enterococci

As previously noted, enterococci exhibit significant resistance to a wide variety of antimicrobial agents. This resistance is almost certainly relevant in most natural ecological settings in which enterococci dwell. As normal commensals of the human gastrointestinal tract, enterococci are routinely exposed to a myriad of antibiotics in the course of contemporary medical treatment, and enterococcal resistance plays a key role in the ecological dynamics that occur during and after antibiotic therapy. In addition, their resistance has confounded the best efforts of contemporary medicine to cope with infections caused by enterococci.

Intrinsic resistance—that which is encoded within the core genome of all members of the species—differs from acquired resistance, in that the latter is present in only some members of the species and is obtained via the horizontal exchange of mobile genetic elements (or via selection upon antibiotic exposure). A great deal of effort has been devoted to understanding the molecular mechanisms of resistance in enterococci. This has resulted in identification of determinants that specify resistance for many antibiotics, including those that are (or once were) clinically useful as therapeutics to treat enterococcal infections, as well as those to which enterococci, as commensals of humans, are incidentally exposed in the course of therapy for infections caused by other bacteria. In many cases, this research has led to the development of an understanding of the regulation and biochemical activities of the resistance determinants, and, in selected cases, has provided insight into the consequences of antibiotic resistance on the biological fitness of enterococci. This section will provide an overview of mechanisms of resistance that have been examined in the past 10 years.

Glycopeptide resistance

The glycopeptides vancomycin, teicoplanin, and newer derivatives, are used to treat serious infections due to resistant Gram-positive bacteria. Most Gram-negative bacteria are not susceptible to glycopeptides because their outer membrane prevents access to the peptidoglycan targets located in the periplasmic space. Glycopeptides inhibit bacterial growth by interfering with peptidoglycan biosynthesis. The antibiotics form complexes with the

D-Ala-D-Ala peptide termini of peptidoglycan precursors on the outer surface of the cell, which prevents the cell wall biosynthetic enzymes (*i.e.*, the PBPs) from using them as substrates for transglycosylation and transpeptidation and, hence, impairment of cell wall integrity.

Glycopeptide resistance has been extensively reviewed (Arthur & Courvalin, 1993; Arthur & Quintiliani, Jr., 2001; Courvalin, 2005; Courvalin, 2006; Depardieu, Podglajen, Leclercq, Collatz, & Courvalin, 2007; Jaspan, et al., 2010). The biochemical basis for resistance derives from modification of the antibiotic target. Glycopeptide-resistant enterococci produce altered peptidoglycan precursors in which the D-Ala-D-Ala termini have been modified such that they terminate in either D-Ala-D-lactate or D-Ala-D-Ser. These substitutions reduce the binding affinity of the antibiotics for the peptidoglycan precursors (~1000 fold reduction for D-Ala-D-lac; ~7 fold for D-Ala-D-Ser). The altered precursors can still serve as substrates for the cell wall biosynthetic enzymes to enable the construction of functional peptidoglycan, but the reduced affinity of glycopeptides renders the drugs unable to inhibit cell wall biosynthesis. The capacity to produce the alternative glycopeptide-resistant peptidoglycan precursors is encoded by resistance operons usually encoded on mobile genetic elements (and thus transferable to otherwise susceptible hosts). Specific types of glycopeptide resistance are encoded in the chromosome as part of the core genome of certain enterococcal species.

Overview of genetic mechanisms of glycopeptide resistance

Nine distinct gene clusters conferring glycopeptide resistance have been described in enterococci. These determinants differ from each other both genetically and phenotypically, based on their physical location (encoded on a mobile genetic element or in the core genome); the specific glycopeptides to which they confer resistance (often distinguished operationally as providing resistance to both vancomycin and teicoplanin, or providing resistance to vancomycin but not teicoplanin); the level of resistance they confer; whether resistance is inducible or constitutively expressed; and the type of peptidoglycan precursor that is produced by their gene products. The Van gene clusters encode several functions: (i) a regulatory module, namely a two-component signal transduction system that is responsible for sensing the presence of glycopeptides and activating expression of the resistance genes in inducible Van types; (ii) enzymes that produce the modified peptidoglycan precursors, including enzymatic machinery that is required to produce the appropriate substitute (D-Lac or D-Ser), and a ligase that fuses D-Ala to either D-Lac or D-Ser to make the corresponding dipeptide that can be incorporated into peptidoglycan precursors via the normal biosynthetic machinery of the cell; and (iii) D,D-carboxypeptidases that eliminate any of the normal (unmodified) peptidoglycan precursor synthesized by the natural biosynthetic machinery of the cell, thereby ensuring that nearly all precursors reaching the cell surface are of the modified variety. The Van gene clusters are typically referred to by the names given to the ligases they encode (VanA, VanB, VanC, and so on). The VanA and VanB types are the most common among clinical isolates and have been studied in the greatest detail.

The VanA determinant (Figure 1) confers a high level of resistance to vancomycin and teicoplanin. VanA is typically encoded on Tn*1546* or related transposons, and includes seven open reading frames transcribed from two separate promoters. The regulatory apparatus is encoded by the VanR (response regulator) and VanS (sensor kinase) two-component system, which are transcribed from a common promoter, while the remaining genes are transcribed from a second promoter. Gene products that specify the production of modified peptidoglycan precursors include VanH (dehydrogenase that converts pyruvate to lactate) and VanA (ligase that forms D-Ala-D-Llac dipeptide). The VanX (dipeptidase that cleaves D-Ala-D-Ala) and VanY (D,D-carboxypeptidase) peptidases serve to eliminate the natural peptidoglycan precursors from the cell. The 7th gene, VanZ, is often referred to as an "accessory" function, but its role in resistance is not fully understood.

The VanB locus (Figure 1) confers moderate to high-level resistance to vancomycin, but is not induced by teicoplanin. VanB is usually acquired onTn*5382*/Tn*1549* type transposons, which occur on plasmids or in the chromosome of the host. The genetic organization of VanB is similar to that of VanA, in that it contains two distinct promoters transcribing seven open reading frames, but there are some significant differences. For

example, although VanB encodes a two-component system (named $VanR_B$ and $VanS_B$), this signaling system is considerably different from that encoded in VanA. VanB encodes homologs of VanH and the D-Ala-D-Ala ligase (encoded by VanB), as well as the peptidases (VanX and VanY). However VanB lacks a homolog of VanZ, and instead encodes a protein named VanW, whose role in resistance is not fully understood.

Regulation of glycopeptide resistance

Expression of vancomycin resistance is controlled via the VanR/VanS two-component signal transduction system, shown in Figure 1. The VanS sensor kinase is thought to recognize a (poorly defined) stimulus that signals the presence of vancomycin in the environment. VanS thereby becomes activated and autophosphorylates a conserved histidine residue on the cytoplasmic side of the protein. That phosphoryl group can be transferred to a conserved aspartate residue on the VanR response regulator, which leads to VanR dimerization, enhanced VanR binding to the 2 promoters located in the Van locus, and consequently, an increased transcription of both the Van resistance genes as well as the regulatory genes (Depardieu, Courvalin, & Kolb, 2005). Additionally, there is substantial evidence that VanS serves as a phosphatase for VanR under non-inducing conditions, to prevent activation of Van expression via spurious phosphorylation from other sensor kinases in the cell (cross-talk), or via autophosphorylation of VanR from acetyl-phosphate (Depardieu, Courvalin, & Kolb, 2005). As a result, the phosphatase activity of VanS is critical to maintain the signaling pathway in the off state in the absence of an inducing antibiotic. Mutations that impair the phosphatase activity of VanS (or remove VanS completely) lead to constitutive expression of the resistance genes (Arthur & Quintiliani, Jr., 2001). This is now known to be true for many of the Van clusters (Depardieu, Kolbert, Pruul, Bell, & Courvalin, 2004; Panesso, et al., 2010). In fact, constitutively resistant mutants that carry lesions in VanS can be isolated from patients during glycopeptide therapy. For example, examination of successive isolates of *E. faecium* obtained from a patient suffering from an infection with a VanB strain revealed that a short deletion in the $VanS_B$ kinase led to the loss of phosphatase activity and constitutive glycopeptide resistance (Depardieu, Courvalin, & Msadek, 2003).

Although both VanA and VanB rely on two-component signaling systems to control Van expression, it is clear that there are important differences between these regulatory systems. For example, the VanS and $VanS_B$ sensor kinases exhibit relatively little sequence identity in the N-terminal portion that serves as the site of stimulus recognition. In fact, the amino acid sequence of the predicted extracellular ligand-binding domain of VanS is short, and is likely to comprise only a short loop that connects the two transmembrane helices outside the membrane, which suggests that VanS belongs to the intramembrane-sensing family of sensor kinases (Mascio, Alder, & Silverman, 2007), whereas the predicted extracellular domain of $VanS_B$ is substantially larger and likely constitutes an independently-folded extracellular domain that serves to recognize cognate signals. Given the distinct architecture of these two sensor kinases, it seems plausible that they recognize and respond to different molecular signals to trigger kinase activation and expression of the resistance genes. In fact, this predicted difference in ligand binding—and consequently, in the inducibility of the signaling system—underlies the difference in teicoplanin susceptibility of enterococci that contain VanA vs. those that contain VanB. Although the molecular identity of the actual inducing signal(s) remain unclear, the VanA resistance genes are induced by the presence of both vancomycin and teicoplanin (thereby conferring resistance to both), but the VanB resistance genes are only induced by vancomycin—hence, VanB strains remain susceptible to teicoplanin. Of note, $VanS_B$ can acquire mutations of various types that lead to constitutive expression of the resistance genes or to inducibility by teicoplanin, thereby altering the phenotype of such mutants carrying the VanB locus.

Regulation by host factors?

Some evidence suggests that one or more sensor kinases encoded in the genome of the enterococcal host can contribute to the regulation of the Van resistance genes. For example, $VanR_B$-dependent gene expression remains inducible even in the absence of $VanS_B$ (Baptista, Rodrigues, Depardieu, Courvalin, & Arthur, 1999), suggesting that another sensor kinase can phosphorylate and activate $VanR_B$. Similarly, the VanE cluster in *E.*

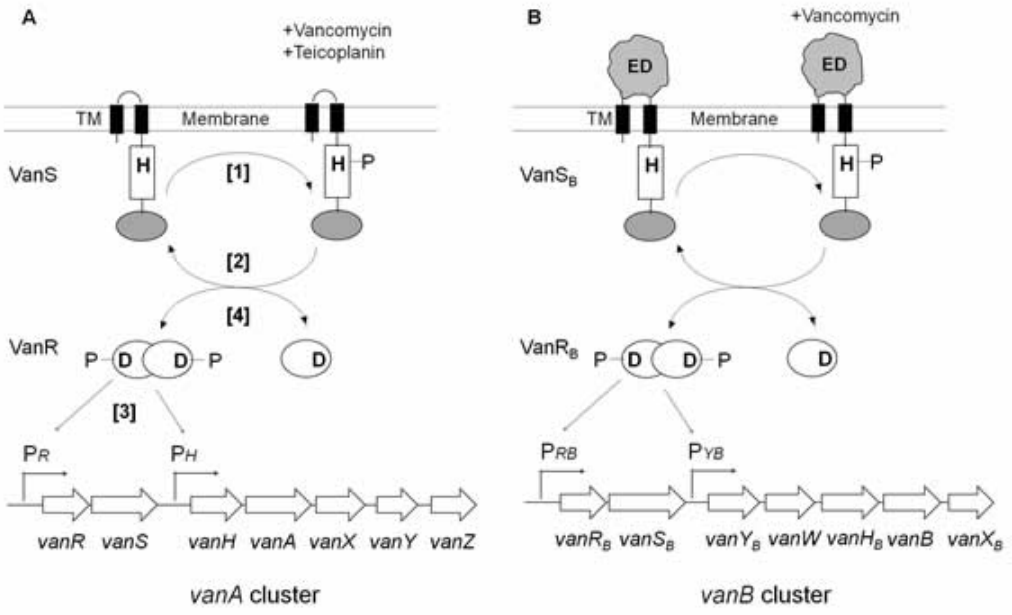


Figure 1. Regulation of vancomycin resistance gene clusters. Comparison of VanA regulation (panel A) and VanB regulation (panel B). The VanS (or VanSB) sensor kinases are anchored in the cytoplasmic membrane by two transmembrane segments (TM) that flank the predicted sensory input domain. VanS (which is inducible by both vancomycin and teicoplanin) contains only a short extracellular loop, and may receive activating signals in, or immediately adjacent to, the membrane. VanSB (which is inducible by vancomycin, but not teicoplanin) contains a large extracellular domain (ED) that likely serves as the ligand-recognition domain. In both cases, the presence of the appropriate antibiotic stimulus leads to the activation of kinase activity and ATP-dependent autophosphorylation on a highly conserved His residue [1]. This phosphoryl group is transferred to the VanR (or VanRB) response regulator [2], which leads to dimerization, enhanced binding to DNA, and activation of transcription from the two promoters found in the Van gene cluster [3]. In the absence of inducing stimuli, VanS (VanSB) serves as a phosphatase to ensure that VanR (VanRB) remains in the inactive state [4] and Van expression is off. Figure is adapted from (Arthur & Quintiliani, Jr., 2001) with modifications.

faecalis encodes a $VanS_E$ kinase that is predicted to be nonfunctional due to a premature stop codon (Abadía Patiño, Courvalin, & Perichon, 2002), but such resistance is nevertheless inducible by vancomycin (Foucault, Depardieu, Courvalin, & Grillot-Courvalin, 2010). Such findings suggest that enterococci encode endogenous two-component signaling systems whose natural function is to monitor the integrity of the cell wall for perturbations, and activate appropriate adaptive responses to ensure cell wall maintenance; and further, that the glycopeptide resistance gene cassettes have managed to exploit these endogenous systems to assist in the regulation of glycopeptide resistance. Other host factors may also play a role in regulation of Van expression. For example, expression of the VanE vancomycin resistance genes may be influenced by the alteration of DNA supercoiling in *E. faecalis* (Paulsen, et al., 2003).

The expanding Van alphabet

While the VanA- and VanB-type vancomycin resistance clusters continue to be the predominant forms that account for vancomycin resistance in hospitals, new Van resistance gene clusters have been recently described, which brings the number of known gene clusters capable of conferring Van resistance to nine. Lebreton and colleagues (Lee, Huda, Kuroda, Mizushima, & Tsuchiya, 2003) recently described such a new gene cluster, named VanN, that specifies incorporation of D-Ala-D-Ser at the terminus of the peptidoglycan precursors. VanN joins other recently described Van clusters (Boyd, Willey, Fawcett, Gillani, & Mulvey, 2008; Xu, et al., 2010) known to specify incorporation of either D-Ala-D-Ser (VanC, VanE, VanG, and VanL types) or D-Ala-D-Lac (VanA, VanB, VanD, and VanM types) into peptidoglycan precursors.

Fitness cost of vancomycin resistance

Despite the complex mechanism that underlies glycopeptide resistance, resistant enterococci have disseminated worldwide, suggesting that resistance imposes little or no biological cost to the bacteria. This hypothesis was carefully examined by using pairs of isogenic enterococcal strains with specific mutations, to evaluate the fitness cost of vancomycin resistance during growth *in vitro* and *in vivo* (Franke & Clewell, 1981). The investigators found that expression of vancomycin resistance imposed a significant fitness cost, both when expression is induced by the antibiotic and when expression is constitutive due to mutation of the regulatory apparatus. However, uninduced vancomycin resistance did not impose a measurable fitness cost. Thus, these results offer a strong evolutionary rationale for the tight regulation of vancomycin resistance by the VanS/VanR two-component signaling system found in Van gene clusters.

Alternative mechanism of glycopeptide resistance

A novel mechanism of glycopeptide resistance has been described in laboratory-selected vancomycin-resistant mutants of *E. faecium* (Cremniter, et al., 2006). This mechanism is unrelated to that encoded by the Van gene clusters (namely, those with production of peptidoglycan precursors containing D-Lac or D-Ser substitutions). The investigators selected highly resistant mutants *in vitro* and performed extensive analysis of peptidoglycan structure in the mutants. Their analysis revealed that the beta-lactam insensitive L,D-transpeptidase pathway (discussed in more detail below, under *Ampicillin resistance*) was activated. This alternative transpeptidase (named Ldt_{fm}) is capable of crosslinking enterococcal peptidoglycan using the L-Lys found at the 3rd position of the peptide stem (rather than the D-Ala found at position 4, as is typical of most PBPs). The investigators found that a cryptic D,D-carboxypeptidase was activated in the glycopeptide-resistant mutants, whose activity resulted in production of peptidoglycan peptide stem precursors that are tetrapeptides (lacking the terminal D-Ala), rather than pentapeptides. Such precursors are not substrates for binding by glycopeptide antibiotics, but can be cross-linked by the Ldt_{fm} transpeptidase. However, it remains unknown whether this mechanism of glycopeptide resistance is relevant in clinical isolates.

Daptomycin resistance

Daptomycin is a lipopeptide antibiotic with potent *in vitro* bactericidal activity against Gram-positive bacteria. The mechanism of antimicrobial action for daptomycin has not been unequivocally established, but is thought to involve calcium-dependent insertion into the cytoplasmic membrane followed by membrane depolarization, release of intracellular potassium ions, and rapid cell death (Alborn, Jr., Allen, & Preston, 1991; Matsumura & Simor, 1998; Silverman, Perlmutter, & Shapiro, 2003). Because its mechanism of action is distinct from those of other antibiotics, daptomycin is useful for treatment of infections that are caused by multidrug-resistant Gram-positive strains. Daptomycin resistance has been observed in clinical isolates following daptomycin therapy, typically as a result of mutations in chromosomal genes. In *Staphylococcus aureus*, resistance is associated with mutations in genes encoding proteins such as MprF, a lysylphosphatidylglycerol synthetase; YycG, a sensor histidine kinase; and RpoB and RpoC, the β and β′ subunits of RNA polymerase (Galimand, et al., 2011).

Daptomycin-nonsusceptible clinical *E. faecium* strains have been described (Muller, Le Breton, Morin, Benachour, Auffray, & Rincé, 2006). The investigators determined that these strains did not carry mutations in homologs of genes known to confer nonsusceptibility to daptomycin in *S. aureus* (*yycG*, *mprF*, *rpoB*, *rpoC* were evaluated in this study), which suggests the existence of one or more novel mechanisms of daptomycin resistance in enterococci. However, the genes responsible for resistance were not identified.

Recent studies have begun to explore the genetic basis of daptomycin resistance in enterococci (Arias, et al., 2011). The genomes of a pair of *E. faecalis* strains isolated from the same patient before and after daptomycin therapy were sequenced to identify polymorphisms contributing to resistance (Arias, et al., 2011). In that study, unique-sequence polymorphisms were found in *cls*, *gdpD* (both thought to be involved in phospholipid metabolism) and *liaF*, but no polymorphisms were found in homologs of the genes identified in daptomycin-resistant *S. aureus* isolates. Follow up analysis with *in vitro* selection for daptomycin-resistant variants of the original susceptible strain led to the identification of mutations in *liaF* and *gdpD*. Importantly, the daptomycin-resistant phenotype was determined to be a consequence of the identified mutations, as site-directed mutagenesis to recapitulate these mutations in an otherwise daptomycin-susceptible host conferred enhanced daptomycin resistance, demonstrating that mutations in these genes confer resistance. Similarly, in a clinical strain pair of *E. faecium* recovered from a patient both before and after daptomycin therapy, a polymorphism in *cls* was identified (but not in *liaFSR* or *gdpD*) in the daptomycin-resistant derivative. Changes in *cls*, *liaF*, *liaS*, or *liaR* were also identified in other daptomycin-resistant clinical isolates of enterococci, which suggests that such mutations play a key role in the development of daptomycin resistance *in vivo*. Development of daptomycin resistance appeared to be associated with profound ultrastructural changes in the cell envelope and septal apparatus, although it remains unclear if these changes are functionally important for resistance or merely an incidental consequence of the mutations. LiaF is part of the three-component LiaFSR regulatory system, which is known to coordinate the response of the cell envelope to antibiotics and antimicrobial peptides in some Gram-positive bacteria, which suggests that perturbations in the activity of this signaling system may alter envelope properties in a such a way that daptomycin can no longer interact with, or insert into, the membrane efficiently.

In parallel studies, Palmer and coworkers systematically selected daptomycin-resistant variants of *E. faecalis in vitro* (Palmer, Kos, & Gilmore, 2010) in a stepwise manner, and characterized the order of appearance of mutations that correspond to increased resistance to daptomycin. Whole-genome sequence comparison identified mutations in seven genes, including *cls*, *rpoN*, and additional genes whose cellular functions in other contexts have not been established. Transfer of the *cls* mutant allele to a susceptible *E. faecalis* strain conferred enhanced resistance to daptomycin, which unambiguously proved that the *cls* mutation is sufficient for resistance. Time-resolved analysis of the emergence of mutations during daptomycin exposure revealed that *cls* mutations appeared early in multiple independent selections, which highlights its importance. Additional daptomycin-resistant mutants were obtained that lacked such *cls* mutation, which shows that alternative paths to daptomycin resistance also exist. Collectively, the results from these two studies suggest that, while the underlying mechanisms are genetically distinct from those identified in *S. aureus*, a role for altered membrane phospholipid composition and/or surface properties in both staphylococcal and enterococcal daptomycin resistance is likely.

Aminoglycoside resistance

Aminoglycosides act by binding to the 16S rRNA of the 30S ribosomal subunit and interfering with protein synthesis. Enterococci generally exhibit a moderate level of intrinsic aminoglycoside resistance that has been attributed to poor uptake of antibiotics. For example, analysis of selected mutants that exhibited enhanced gentamicin resistance *in vitro* suggested that impaired uptake of gentamicin can contribute directly to enhanced resistance (Aslangul, et al., 2006), although the mutations or genes responsible for the alteration in uptake were not unambiguously identified. However, some evidence suggests that other mechanisms may contribute to, or even be primarily responsible for, intrinsic resistance of some enterococci to aminoglycosides.

Moderate species-specific intrinsic resistance to aminoglycosides in *E. faecium* is enhanced by a chromosomally encoded rRNA methyltransferase, EfmM (Galloway-Peña, Roh, Latorre, Qin, & Murray, 2012) that uses S-adenosyl methionine as a methyl donor to methylate a specific residue on 16S rRNA, in the context of the 30S ribosomal subunit. The inactivation of *efmM* in *E. faecium* increases susceptibility to the aminoglycosides kanamycin and tobramycin, and conversely, expression of a recombinant version of *efmM* in *Escherichia coli* confers enhanced resistance to these drugs. Addition of the 5-methyl to C1404 sterically hinders aminoglycoside binding. In addition, a chromosomally encoded 6′-N-aminoglycoside acetyltransferase (*aac(6′)-Ii)* confers low-level intrinsic resistance (Costa, Galimand, Leclercq, Duval, & Courvalin, 1993). The physiological effects of these two factors seems to be additive, in the sense that mutation of both genes led to the largest reduction in resistance. However, it remains unclear if aminoglycoside resistance is the primary function of EfmM. The position of m5C1404 at the junction between the ribosomal A-site and the P-site in 16S rRNA suggests that this modification might also play a more basic role in protein synthesis by influencing codon-anticodon interactions.

High-level resistance to aminoglycosides (HLGR) is conferred by a mechanism distinct from those described above, and importantly, abolishes the synergistic bactericidal activity of aminoglycosides in combination with cell-wall–active agents that are important in the treatment of severe enterococcal infections, such as endocarditis. HLGR is usually acquired on a mobile element that encodes an aminoglycoside-modifying enzyme. Such enzymes can be phosphotransferases (APHs) that use ATP to phosphorylate a hydroxyl group on the antibiotic, acetyltransferases (AACs) that use acetyl-CoA to acetylate an amino group on the antibiotic, or nucleotidyltransferases (ANTs) that use ATP to adenylylate a hydroxyl group on the antibiotic. HLGR is most often associated with members of the APH(2′′)-I phosphotransferase family or the bifunctional AAC(6′)-Ie-APH(2′′)-Ia family that are encoded on various transposons or conjugative plasmids (reviewed in (Kak, Donabedian, Zervos, Kariyama, Kumon, & Chow, 2000)).

Rifampicin resistance

Rifampicin inhibits bacterial growth by binding to the beta subunit of RNA polymerase (RpoB) and preventing initiation of transcription (Wehrli, Knüsel, Schmid, & Staehelin, 1968). Rifampicin has been used for decades as part of an antibiotic cocktail to treat infections caused by *Mycobacterium tuberculosis*, and has recently found increasing use in the treatment of staphylococcal infections associated with indwelling medical devices, such as artificial joints. Most resistance to rifampicin results from mutations of specific sites in the gene encoding the beta subunit of the RNA polymerase, which reduces the affinity of rifampicin for the polymerase. Mutations in RpoB responsible for rifampicin resistance have been identified in numerous and diverse species of bacteria. Additionally, enzymatic inactivation of rifampicin has been observed in a handful of cases.

Although rifampicin has not been used extensively to treat enterococcal infections, acquired resistance to rifampicin is nonetheless common in enterococci—nearly 79% of 71 clinical isolates were found to exhibit rifampicin resistance (Andrews, Ashby, Jevons, Marshall, Lines, & Wise, 2000), as well as >57% of a diverse collection of isolates from six countries in Europe (Lautenbach, Schuster, Bilker, & Brennan, 1998). Presumably this is at least partially a consequence of commensal enterococci being exposed to rifampicin during treatment for non-enterococcal infections, but other as-yet-unknown factors may contribute to the occurrence of resistant enterococcal isolates as well. In *E. faecium*, substitutions in RpoB are associated with rifampicin resistance, and most of the identified RpoB polymorphisms have been previously implicated in conferring resistance in other species of bacteria. The biological cost of rifampicin resistance is variable, depending on the particular mutation in RpoB, as well as other potential compensatory mutations that may occur elsewhere in the genome (Enne, Delsol, Roe, & Bennett, 2004).

Spontaneous rifampicin-resistant mutants of *E. faecalis* and *E. faecium* are readily isolated *in vitro* (Kristich, Little, Hall, & Hoff, 2011). Mutations were identified in the *rpoB* gene of all such mutants at sites known to be associated with resistance to rifampicin in other species of bacteria. For two particular mutants, confirmation that the *rpoB* point mutations are indeed responsible for rifampicin resistance was obtained by expressing the

mutant alleles in an otherwise rifampicin-susceptible host. One unexpected observation was that some RpoB mutations led to an alteration in cephalosporin resistance in an allele-specific manner. In particular, the *rpoB* H486Y mutation conferred a substantially enhanced resistance to cephalosporins in multiple lineages of *E. faecalis,* as well as in *E. faecium*, whereas other Rif-resistant substitutions in *rpoB* did not affect cephalosporin resistance. The mechanistic basis for this observation is not known, but the *rpoB* H486Y substitution may alter rates of transcription of genes that contribute to intrinsic cephalosporin resistance in enterococci.

Rifampicin resistance in some isolates of *E. faecium* can be reversed by inclusion of subinhibitory concentrations of daptomycin. This phenomenon could not be explained by 1) alteration of rifampicin transport into, or efflux from, the cell by daptomycin; 2) the direct inactivation of rifampicin; nor 3) mutations at the rifampicin-binding site in *rpoB* (Reynolds & Courvalin, 2005). The molecular explanation for this effect remains unknown and authors speculate that there may be another mechanism for rifampicin resistance besides the two known mechanisms (namely, mutation in the *rpoB* gene and rifampicin inactivation). This third mechanism has been dubbed "daptomycin-reversible resistance," and further investigation will be needed to define its basis.

Quinolone resistance

Quinolones generally exhibit only moderate activity against enterococci. Quinolones inhibit the growth of bacteria by interfering with DNA replication, specifically by binding to the type II topisomerases that control DNA supercoiling (DNA gyrase and DNA topoisomerase IV) and inhibiting their function, leading to lethal double-strand breaks in the DNA. Quinolone resistance in many species of bacteria occurs via mutations in the "quinolone resistance determining regions" of the genes that encode gyrase and topoisomerase IV. These mutations prevent efficient binding of the antibiotic to the enzyme, which enables DNA replication to continue despite the presence of the antibiotic. Such mutations have been observed in clinical and lab-derived quinolone-resistant isolates of enterococci (Kak, Donabedian, Zervos, Kariyama, Kumon, & Chow, 2000; Oyamada Y. , Ito, Inoue, & Yamagishi, 2006; Palmer, Daniel, Hardy, Silverman, & Gilmore, 2011; Werner, Fleige, Ewert, Laverde-Gomez, Klare, & Witte, 2010), and presumably act to confer enhanced quinolone resistance through a similar mechanism.

A second mechanism known to contribute to quinolone resistance in other species of bacteria is efflux of the antibiotic out of the cell. Such efflux is often a function of pumps with relatively broad or nonspecific substrate specificities, which are sometimes referred to as multidrug-resistance efflux pumps (MDRs). Genes encoding MDRs are usually found on the bacterial chromosome. Although the primary physiological functions for most MDRs remain unclear, these proteins are known to actively transport toxic compounds out of the cell. The genome of *E. faecalis* V583 is predicted to encode 34 MDRs (Davis, et al., 2001), which suggests that drug efflux may play an important role in antibiotic resistance. Two of these pumps have been experimentally implicated in promoting quinolone resistance. The first is EmeA, a homolog of *Staphylococcus aureus* MDR NorA. Mutation of EmeA resulted in modest increases in susceptibility to several compounds, including quinolones (Jung, et al., 2010), and treatment of wild-type *E. faecalis* OG1RF with known MDR inhibitors (reserpine, lansoprazole, and verapamil) inhibits the efflux of the quinolone norfloxacin, as well as the toxic compound ethidium bromide. The second pump is EfrAB, an ABC-type transporter that enhances resistance to a variety of structurally unrelated compounds, including quinolones, when expressed in *E. coli* (Lefort, Saleh-Mghir, Garry, Carbon, & Fantin, 2000). Its function in enterococci was not investigated.

A new mechanism of quinolone resistance has been identified—protection of DNA gyrase and topoisomerase IV from inhibition by quinolones. The activity is provided by members of the Qnr protein family that were originally identified in enterobacteria as a transmissible type of quinolone resistance (Mascher, Helmann, & Unden, 2006). The natural function of Qnr proteins remains unclear. These proteins are characterized by tandem pentapeptide repeats, and homologs of Qnr appear to be encoded in the genomes of various bacteria. Inactivation of a homolog of Qnr identified in the genome of *E. faecalis*, comprised of 42 predicted pentapeptide repeats, resulted in a modest decrease in resistance to fluoroquinolones. Overexpression of the corresponding

gene yielded an increase in resistance. Furthermore, expression of the *E. faecalis* gene in heterologous organisms, including *S. aureus* and *Lactococcus lactis*, increased the level of quinolone resistance in those hosts (Arsène & Leclercq, 2007). Purified EfsQnr inhibited the ATP-dependent DNA supercoiling activity of *E. coli* gyrase (Hellinger, Rouse, Rabadan, Henry, Steckelberg, & Wilson, 1992), suggesting that EfsQnr may protect *E. faecalis* from the effects of quinolones by modulating the action of gyrase in cells.

Macrolide, lincosamide, and streptogramin resistance

Macrolides, lincosamides, and streptogramin antibiotics inhibit protein synthesis by binding to the 50S subunit of the ribosome. Macrolides and lincosamides are not used to treat enterococcal infections, but resistance to them is nonetheless widespread (Jonas, Murray, & Weinstock, 2001). The most common form of acquired resistance to macrolides is production of an enzyme that methylates a specific adenine in the 23S rRNA of the 50S ribosomal subunit, which reduces the binding affinity of the macrolide for the ribosome. This modification also reduces the binding of lincosamide and streptogramin B antibiotics to the ribosome. The responsible enzyme is typically encoded by the *ermB* gene, and the phenotype is often referred to as MLS_B. An efflux pump, encoded by the transferrable *mefA* gene, is also known to pump macrolides out of the cell, but confers a lower level of resistance than *ermB* (Clancy, et al., 1996).

E. faecalis and *E. faecium* are known to exhibit different intrinsic susceptibilities to quinupristin-dalfopristin (Q-D), members of the streptogramin family that act synergistically. *E. faecalis* is sufficiently intrinsically resistant that these antibiotics cannot be used therapeutically, whereas *E. faecium* is usually susceptible. The molecular basis for this difference appears to stem from the existence of a chromosomally encoded putative ABC transporter in the *E. faecalis* genome, named Lsa (198). Lsa is encoded in the genome of all isolates of *E. faecalis* evaluated (n=180), but in none of the genomes of other enterococci (n=189). Disruption the *lsa* gene in *E. faecalis* OG1RF resulted in a >40-fold decrease in MIC to Q-D. In addition, expression of the *E. faecalis* V583 *lsa* gene in otherwise susceptible *E. faecium* led to a 6-fold increase in MIC. Expression of *lsa* in the heterologous host *Lactococcus lactis* also moderately increased Q-D resistance (Singh & Murray, 2005). Further support for a role of Lsa in Q-D resistance stems from the observation that clinical isolates of *E. faecalis* that are susceptible to lincosamides and dalfopristin harbor mutations in *lsa* that result in premature stop codons (Dina, Malbruny, & Leclercq, 2003). However, the influence of Lsa on efflux or transport of the antibiotics has not been directly evaluated. Such studies will be helpful in determining the mechanism by which Lsa provides resistance, as the protein itself contains ATP-binding domains characteristic of ABC transporters, but lacks identifiable transmembrane sequences that would be expected of an authentic efflux pump. As such, the molecular mechanism of Lsa action remains unknown. Acquired resistance to Q-D has emerged in *E. faecium*, and is mediated by members of the streptogramin acetyltransferase family of enzymes that acetylate streptogramin A (such as VatH), and by the Vga genes, which encode an ABC transporter that presumably function to export the antibiotic from the cell (Kak & Chow, 2002).

Beta-lactam resistance

Antibiotics in the beta-lactam family inhibit bacterial growth by serving as suicide substrates for the D,D-transpeptidases (also known as penicillin-binding proteins, or PBPs) that catalyze cross-linking of peptidoglycan peptide side chains during the synthesis of mature peptidoglycan. Once modified by a beta-lactam antibiotic, PBPs are inactivated, thereby preventing continued cell wall synthesis. Enterococci exhibit intrinsic nonsusceptibility to beta-lactam antibiotics, but the extent of nonsusceptibility varies among the different classes of beta-lactams and between enterococcal species: penicillins have the most activity against enterococci (and *E. faecium* is inherently a bit more resistant than *E. faecalis*), carbapenems slightly less, and cephalosporins exhibit the least activity. This spectrum of activity is reflected in the utility of these drugs for treatment, insofar as ampicillin remains an effective therapy for susceptible enterococcal infections, but cephalosporins are completely

ineffective against enterococci. In fact, prior use of cephalosporins is a major risk factor for the acquisition of an enterococcal infection (reviewed in (Shepard & Gilmore, 2002)).

The intrinsic nonsuceptibility of enterococci to beta-lactams involves the production of the low-affinity class B penicillin-binding protein 5 (Pbp5), an ortholog of the low-affinity Pbp2a expressed by methicillin-resistant isolates of *Staphylococcus aureus* (Gonzales, Schrekenberger, Graham, Kelkar, DenBesten, & Quinn, 2001). Due to its relatively low affinity for beta-lactams, the chromosomally encoded Pbp5 is capable of carrying out peptidoglycan synthesis at concentrations of beta-lactam antibiotics that saturate all of the other enterococcal PBPs (Canepari, Lleò, Cornaglia, Fontana, & Satta, 1986), and therefore is required for beta-lactam resistance (Arbeloa, et al., 2004; Rice L. B., Carias, Rudin, Lakticová, Wood, & Hutton-Thomas, 2005). Pbp5 is required for both the intrinsic beta-lactam resistance traits of enterococci, such as their intrinsic cephalosporin resistance, as well as for the acquired (enhanced) resistance to members of the beta-lactam family (such as ampicillin), to which enterococci are otherwise clinically susceptible. Below, we discuss resistance to two different families of beta-lactam antibiotics (cephalosporins and ampicillin) in more detail.

Intrinsic cephalosporin resistance

E. faecalis and *E. faecium* are naturally (intrinsically) resistant to cephalosporins. This trait has been known for decades and is encoded by chromosomal determinants in the core genome of these organisms, but its molecular basis remains incompletely understood. Thus far, in *E. faecalis*, a handful of genetic determinants have been shown to be required for intrinsic cephalosporin resistance: the low-affinity penicillin-binding protein Pbp5; a two-component signal transduction system, CroRS; a transmembrane Ser/Thr kinase, IreK; and one of the early enzymes involved in synthesis of peptidoglycan precursors, MurAA. The role of these determinants has been best studied in *E. faecalis*, although it appears likely that similar mechanisms are present in in *E. faecium*.

pbp5

Genetic studies on isogenic mutants of *E. faecalis* and *E. faecium* provide clear evidence of a requirement for Pbp5 in intrinsic cephalosporin resistance (Arbeloa, et al., 2004; Rice L. B., Carias, Rudin, Lakticová, Wood, & Hutton-Thomas, 2005). Deletion mutants that lack *pbp5* exhibit large reductions in the level of cephalosporin resistance. The deletion mutants also exhibit a reduction in resistance to the non-cephalosporin beta-lactam, ampicillin, although the magnitude of the reduction is more modest (especially for *E. faecalis*). High-molecular-weight PBPs are often categorized as Class A (bifunctional, exhibiting both transglycosylase and transpeptidase activities) and Class B (monofunctional, exhibiting transpeptidase activity but lacking transglycosylase activity). As a Class B PBP, Pbp5 contains a transpeptidase domain, but lacks a transglycosylase domain, which is necessary for the initial polymerization of the disaccharide moiety of peptidoglycan precursors. Therefore, even though Pbp5 is capable of synthesizing crosslinks between the peptide side chains of peptidoglycan, Pbp5 must cooperate with one or more transglycosylases for cell wall biosynthesis. Candidate transglycosylase partners include the three bifunctional Class A enterococcal PBPs (or in principle, monofunctional transglycosylases analogous to Mgt of *Staphylococcus aureus*, although no examples have yet been identified in enterococci). Analysis of isogenic deletion mutants lacking one or more Class A PBPs in both *E. faecalis* and *E. faecium* revealed that Pbp5 cooperates with one or both Class A PBPs, either PbpF or PonA, to permit growth in the presence of cephalosporins. The third Class A PBP (PbpZ) encoded by these organisms is unable to provide transglycosylase activity in the presence of cephalosporins. Triple mutants of either *E. faecalis* or *E. faecium* that lack all three class A PBPs are viable (although susceptible to cephalosporins), which indicates that as-yet-unidentified additional transglycosylases capable of peptidoglycan polymerization must exist. Given that double mutants lacking *pbpF* and *ponA* are susceptible to cephalosporins, it appears that these unidentified transglycosylases do not have the capacity to participate in functional interactions with Pbp5. Of note, while much of the PBP deletion analysis indicates substantial similarities in the underlying mechanisms of Pbp5 function in *E. faecalis* and *E. faecium*, deletion of the Class A PBPs in *E. faecium* revealed an unexpected dissociation between the expression of resistance to ceftriaxone and ampicillin that was not observed in *E.*

faecalis (Rice L. B., Carias, Rudin, Lakticová, Wood, & Hutton-Thomas, 2005), suggesting that Pbp5-mediated crosslinking of peptidoglycan in *E. faecium* was differentially susceptible to beta-lactams, depending upon partner glycosyltransferase.

croRS

The genome of *E. faecalis* V583 encodes 17 two-component signal transduction systems (TCSs) (Hancock & Perego, 2004). Systematic inactivation of these TCSs and phenotypic characterization of the mutants revealed that the inactivation of CroRS rendered *E. faecalis* susceptible to extended spectrum cephalosporins, but not to a panel of antibiotics that perturb other cellular processes (Hartmann, et al., 2010). These results are consistent with studies in another lineage of *E. faecalis*, in which deletion of the genes encoding the CroRS TCS resulted in a loss of resistance to ceftriaxone (Comenge, et al., 2003). Further analysis of the deletion mutant revealed that the loss of CroRS function did not alter the expression of Pbp5, peptidoglycan precursor production, or peptidoglycan crosslinking. The CroRS TCS appeared to function according to conventional models for TCSs, in that the CroS kinase could autophosphorylate itself in an ATP-dependent manner, followed by the transfer of the phosphoryl group to the CroR response regulator. The CroR response regulator contains a functional DNA binding domain, which suggests that transcriptional remodeling is necessary for adaptation to the stress imposed by cephalosporins. However, only a few genes controlled directly by CroR have been identified thus far. These include *salB*, encoding a secreted protein that does not contribute to cephalosporin resistance (Murray, 1990), *croRS* itself (Murray, 1990), and genes that encode a putative glutamine transporter (Lebreton, et al., 2011) with no obvious connection to cephalosporin resistance. Treatment of *E. faecalis* cells with a panel of cell-wall-active antibiotics resulted in induction of a CroR-dependent promoter, but nothing more is known about the nature of the physiological signal(s) that influences CroS kinase regulation of cephalosporin resistance.

ireK

In addition to the CroRS TCS, a second signal transduction protein (IreK) is required for cephalosporin resistance in *E. faecalis*. IreK exhibits a characteristic bipartite domain architecture that includes a "eukaryotic-type" Ser/Thr kinase coupled, through a putative transmembrane segment, to a series of five repeats of the PASTA domain. Homologs of IreK are nearly universal among the genomes of Gram-positive bacteria. The function of the extracellular PASTA domains is not well understood, but it has been proposed that they bind to peptidoglycan or fragments thereof (Moellering, Jr. & Weinberg, 1971; Squeglia, et al., 2011; Yeats, Finn, & Bateman, 2002), which suggests that IreK could serve as a transmembrane receptor kinase that senses damage or perturbation of the peptidoglycan and initiates a signaling circuit to restore cell wall integrity. Consistent with that view, a homolog of IreK in *Bacillus subtilis* (PrkC) responds to fragments of peptidoglycan released by growing cells, which signals exit from dormancy by *B. subtilis* spores (Shah, Laaberki, Popham, & Dworkin, 2008). Analysis of an *E. faecalis* deletion mutant revealed that IreK is required for intrinsic cephalosporin resistance and for resistance to certain other cell-envelope stresses, such as detergents that are present in bile salts (Kuch, et al., 2012). IreK exhibits protein kinase activity *in vitro*, and its kinase activity is required to promote cephalosporin resistance in *E. faecalis* cells (Kristich, Wells, & Dunny, 2007). As with other members of this kinase family, IreK can catalyze autophosphorylation of threonine residues contained with a specific segment of the kinase domain known as the "activation loop". Phosphorylation at these sites is usually thought to lead to a conformational change, which results in enhanced activity of the kinase (i.e., "activation"). Analysis of site-directed mutants bearing phosphomimetic substitutions at these sites in IreK support the hypothesis that phosphorylation of the IreK activation loop enhances kinase activity *in vivo* and leads to increased cephalosporin resistance (Kristich, Wells, & Dunny, 2007). Other than itself, physiological substrates of IreK in *E. faecalis* cells that are important for cephalosporin resistance have not yet been described.

E. faecalis IreK and its homologs in other low-GC Gram-positive bacteria are encoded immediately adjacent to a gene that encodes a PP2C-type protein phosphatase (called IreP in *E. faecalis*). IreP can dephosphorylate both IreK and substrates of IreK *in vitro*, and analysis of deletion mutants lacking IreP indicate that this activity is

important *in vivo*. IreP mutants exhibit substantial hyperresistance to cephalosporins, a finding which is consistent with hyperactivation of the IreK kinase (Kristich, Wells, & Dunny, 2007). Furthermore, mutants that lack IreP exhibit a large reduction in fitness in the absence of cephalosporins, as compared to wild-type *E. faecalis*, which indicates that uncontrolled activation of cephalosporin resistance mechanisms imparts a significant fitness cost to the cell. The complex regulatory circuitry controlling intrinsic cephalosporin resistance in *E. faecalis* may therefore stem from the fitness cost that is associated with expression of this phenotype.

MurAA

A recent transposon mutagenesis screen in *E. faecalis* revealed a new determinant of intrinsic cephalosporin resistance in enterococci (Vesić & Kristich, 2012). As with most low-GC Gram-positive bacteria, the genome of *E. faecalis* encodes two homologs (annotated as MurAA and MurAB) of the enzyme that catalyzes the first committed step in the synthesis of the peptidoglycan precursor UDP-N-acetylglucosamine 1-carboxyvinyl transferase, which performs PEP-dependent conversion of UDP-N-acetylglucosamine to UDP-N-acetylglucosamine-enolpyruvate. Deletion of *murAA*, but not *murAB*, led to an increased susceptibility of *E. faecalis* to cephalosporins. This enhanced cephalosporin susceptibility does not reflect a general growth or cell-wall synthesis defect of the mutant, because the deletion mutant is not sensitized to antibiotics in general—or even to all antibiotics that inhibit cell wall biosynthesis—but exhibits a loss of resistance specifically for extended spectrum cephalosporins and for fosfomycin (an antibiotic known to target MurA homologs). Chemical genetic analysis revealed synergistic action of ceftriaxone with fosfomycin that was also observed with two strains of *E. faecium*, suggesting that MurAA of *E. faecium* functions in a similar manner to promote cephalosporin resistance. In addition, expression of *murAA*-enhanced cephalosporin resistance in an *E. faecalis* mutant that lacked IreK, which suggests that MurAA may function downstream of IreK in a pathway that leads to cephalosporin resistance. Further genetic analysis revealed that MurAA catalytic activity is necessary, but not sufficient, for this role.

Ampicillin resistance

Modifications in Pbp5 are associated with increased resistance to beta-lactams, such as ampicillin. For example, the Pbp5-encoding gene found in hospital-associated, ampicillin-resistant strains of *E. faecium* differs by ~5% from the corresponding gene in community-associated, ampicillin-susceptible strains (Garnier, Taourit, Glaser, Courvalin, & Galimand, 2000). Most studies that report an association between mutations in Pbp5 and enhanced ampicillin resistance have been performed on non-isogenic clinical isolates, in which unknown factors other than Pbp5 may influence resistance. To circumvent this limitation, Rice and colleagues (Rice, Calderwood, Eliopoulos, Farber, & Karchmer, 1991) used a plasmid-based *pbp5* expression system to explore the impact of specific amino acid substitutions in Pbp5 on ampicillin resistance.

Substitutions that had previously been implicated as contributing to ampicillin resistance in clinical strains conferred modest levels of resistance when expressed from the plasmid-borne *pbp5* in an otherwise-susceptible *E. faecium* host, thereby providing direct evidence of their influence. Combinations of point mutations, especially Pbp5 M485A with a Ser insertion at position 466, yielded substantially enhanced levels of resistance. Furthermore, a correlation was established between the affinity of purified, recombinant Pbp5 mutants for antibiotic binding, with resistance levels provided by these alleles. Further analysis revealed that the chromosomally-encoded *pbp5* determinant could be transferred between strains of *E. faecium* (Rice L. B., 2005) by conjugation, which suggests a mechanism by which high-level ampicillin resistance conferred by mutant *pbp5* alleles could be disseminated among clinical isolates. Although the mechanism of conjugative transfer was not established in that study, a plausible mechanism could involve mobilization mediated by co-integrated enterococcal conjugative plasmids, as recently described in *E. faecalis* (Marshall, Donskey, Hutton-Thomas, Salata, & Rice, 2002). Similar to *E. faecium*, mutations in Pbp5 of clinical isolates of *E. faecalis* may also lead to enhanced resistance to beta-lactam antibiotics, such as imipenem and ampicillin (Oyamada Y. , et al., 2006).

L,D transpeptidation

A multi-step *in vitro* selection process was used to generate a highly ampicillin-resistant mutant of *E. faecium* (Mainardi, et al., 2002). The mutant was found to contain exclusively L-Lys-3-D-Asx-L-Lys cross-links in its peptidoglycan (a product of L,D-transpeptidation, rather than the typical D,D-transpeptidation carried out by PBPs), and peptidoglycan composition was unaffected by the presence of ampicillin. Analysis of peptidoglycan composition of strains with intermediate levels of resistance obtained during the selection process revealed that the balance between D,D-transpeptidation and L,D-transpeptidation influences ampicillin resistance (Manson, Hancock, & Gilmore, 2010). High-level resistance requires elevated activity of a beta-lactam insensitive D,D-carboxypeptidase, which cleaves the peptide stem termini of normal peptidoglycan precursors to generate the substrate for the L,D transpeptidase. The metallo-D,D-carboxypeptidase (DdcY) responsible for cleavage of the peptidoglycan precursors has been identified (183), and its enhanced expression appears to be mediated by mutations that result in activation of a putative cryptic two-component signal transduction system (DdcSR). The enzyme responsible for the formation of the L,D- crosslinks (called Ldt_{fm}) has been identified (Mainardi, Gutmann, Acar, & Goldstein, 1995) and appears to be constitutively expressed in *E. faecium*. Ldt_{fm} is evolutionarily unrelated to the D,D-transpeptidases, in that it uses an active-site Cys nucleophile, rather than a Ser nucleophile. In addition, homologs of Ldt_{fm} are encoded in the genomes of a variety of Gram-positive bacteria (Mainardi, et al., 2005; Mainardi, Gutmann, Acar, & Goldstein, 1995). The normal physiological function of the L,D-transpeptidase is unclear, but it has been proposed to have a role in the maintenance of peptidoglycan structure in stationary-phase cells. Surprisingly, although beta-lactams are usually thought to specifically inhibit the D,D-transpeptidase activity of PBPs, a particular sub-class of the beta-lactam antibiotic family was found to inhibit Ldt_{fm} via covalent modification of the active site Cys (Mainardi, Legrand, Arthur, Schoot, van Heijenoort, & Gutmann, 2000). As noted above, activation of the L,D transpeptidase cross-linking pathway can result in emergence of cross-resistance to beta-lactams and glycopeptides.

Linezolid resistance

Linezolid is a member of the oxazolidinone family of antibiotics developed for use against multidrug-resistant Gram-positive bacteria. Linezolid interferes with bacterial growth by inhibiting protein synthesis through interaction with the translational initiation complex. Resistance to linezolid can be selected *in vitro*, and has also been observed in clinical settings (89, 164). Mutations within the central loop of domain V of 23S rRNA, including a G2576U mutation, are associated with resistance to linezolid, and presumably prevent or reduce binding of the antibiotic to the ribosomal subunit. Analysis of linezolid-resistant *E. faecalis* and *E. faecium* isolates selected during therapy, or obtained from patients following linezolid treatment failure, revealed a direct correlation between the percentage of rRNA genes that carry a G2576U mutation (each genome encodes several copies of the rRNA genes—four in *E. faecalis* and six in *E. faecium*) and the phenotypic level of linezolid resistance, which suggests that the percentage of ribosomes that carry rRNA with the G2576U substitution is the primary determinant for the level of linezolid resistance (Martínez-Martínez, Pascual, & Jacoby, 1998). A similar correlation was observed for linezolid-resistant mutants of *E. faecalis* selected *in vitro* in a recombination-proficient genetic background (Lu, Chang, Perng, & Lee, 2005). Attempts to derive linezolid resistant mutants in a recombination-deficient mutant genetic background did not yield resistant mutants at comparable frequencies. Collectively, these results suggest that recombination between rRNA genes after the emergence of the G2576U mutation may enable the amplification of the level of linezolid resistance in enterococci under the selective pressure imposed by antibiotic treatment.

Enterococci are Generous with Their Genes: Mobile Genetic Elements Enable Facile Horizontal Transfer and Spread of Antibiotic Resistance Determinants Among Enterococci

Gene mobility and exchange have figured prominently in the rise of the enterococcus as a nosocomial pathogen. It is interesting to note that *E. faecalis* OG1X, a fully susceptible strain studied for decades by Don Clewell and others, was found to have no mobile elements and no acquired DNA when its genome was completely sequenced (Bourgogne, et al., 2008). This contrasts sharply with the genome of *E. faecalis* V583, one of the first vancomycin-resistant clinical isolates, in which approximately 25% of its genome consists of acquired DNA (Polidori, et al., 2011). Clearly, existence in clinical settings and the selective pressure of antibiotics in the environment have yielded clinical isolates that have an array of mechanisms to acquire and exchange DNA.

Plasmids

Enterococci were among the first Gram-positive bacteria to have extensively characterized plasmids (Table 1). While a range of different types of plasmids are undoubtedly present in clinical enterococcal strains, the most frequently studied plasmids in the area of antimicrobial resistance are the pheromone-responsive plasmids and the broad host range plasmids (Panesso, Abadía-Patiño, Vanegas, Reynolds, Courvalin, & Arias, 2005). (For more details on plasmid structure and movement, see Extrachromosomal and Mobile Elements in Enteroocci.) The most thoroughly studied pheromone responsive plasmids include pAD1 (Ehrenfeld & Clewell, 1987) and pCF10 (Christie, Korman, Zahler, Adsit, & Dunny, 1987). These plasmids encode responses to small peptide fragments known as pheromones, which leads to the coating of the donor cells with aggregation substance, a sticky protein that promotes clumping of donor and recipient cells and that facilitates the efficient transfer of plasmids (with frequencies as high as 10^{-2}-10^{-1}/recipient CFU). Mating pair aggregation can be observed as the macroscopic clumping of cells in liquid media, and high-frequency transfer is observed both in liquid and on solid media. pAD1 does not encode resistance to any antibiotics, but does encode the production of cytolysin (Segarra, Booth, Morales, Huycke, & Gilmore, 1991), which serves as a virulence factor in all models tested (Clewell, 2007). pCF10 harbors the tet(M) conjugative transposon Tn*925,* which encodes resistance to tetracycline and minocycline Pheromone-responsive plasmids have also been implicated in the transfer of VanB-type vancomycin resistance (Zheng, Tomita, Inoue, & Ike, 2009).

The most completely studied of the pheromone-responsive plasmids have had a host range that appears to be restricted to *E. faecalis*. However, early in the vancomycin resistance era, the transfer of VanA-type vancomycin resistance from *E. faecium* to *E. faecalis* was reported to result from either the transfer of a VanA-encoding pheromone responsive plasmid or through the cointegration of a VanA plasmid and a pheromone-responsive plasmid (Heaton, Discotto, Pucci, & Handwerger, 1996; Hegde, Vetting, Mitchenall, Maxwell, & Blanchard, 2011). Regardless of their origin, pheromone-responsive plasmids described to date do not appear to be able to replicate in non-enterococcal species.

The other major class of enterococcal plasmids associated with the transfer of antimicrobial resistance genes are the so-called "broad host-range," or Inc18 plasmids (Bruand, Chatelier, Ehrlich, & Janniere, 1993). The prototypes for these plasmids are pAMβ1, which encodes resistance to macrolides, and pIP501, which confers resistance to macrolides and chloramphenicol. These plasmids have been shown to transfer to other species (including staphylococci and streptococci), and then to transfer back into enterococci *in vitro*. The frequency of transfer is considerably lower than that seen with pheromone-responsive plasmids, and requires that matings be performed on solid surfaces, as a means of providing cell-cell contact.

Inc18 broad host-range plasmids, or their remnants, have been found in association with a range of different resistance determinants. Tn*5385*, a large, composite, transferable, multi-resistant (streptomycin, tetracycline, gentamicin, erythromycin, β-lactamase) element found in the chromosome of *E. faecalis* CH116 (173), includes

sequences identical to the replication region of pAM β1. Other Inc18 plasmids have been implicated in the transfer of the VanA resistance determinant from *E. faecalis* to *S. aureus* (Zhu, et al., 2010). Inc18 plasmids that mediate vancomycin resistance do not appear to be stable in *S. aureus*, but vancomycin resistance can be stabilized by transposition from the broad host range plasmid to one of the staphylococcal plasmids or to the staphylococcal chromosome.

Table 1. Prototype plasmids and transposons of Enterococci

Plasmid type	Examples	References
Pheromone-responsive	pAD1, pCF10	(Christie, Hammond, Reising, & Evans-Patterson, 1994; Ehrenfeld & Clewell, 1987)
Broad host range (Inc18)	pAMβ1, pIP501	(Qu, Yang, Shen, Wei, & Yu, 2012)
Large, virulence encoding	pLRM23, pLG1	(Le Breton, Muller, Auffray, & Rincé, 2007; Rice & Marshall, 1992)
Transposon type	Examples	References
Composite	Tn*5281*, Tn*5384*, Tn*5385*	(Hong, Hutchings, & Buttner, 2008; Rice L. B., Carias, Hutton-Thomas, & Rudin, 2007; Rice L. B., et al., 2009)
Tn3-family	Tn*917*, Tn*552*, Tn*1546*	(Arthur, Molinas, Depardieu, & Courvalin, 1993; Rowland & Dyke, 1990; Tomich, An, & Clewell, 1980)
Conjugative	Tn*916*, Tn*5382*	(Carias, Rudin, Donskey, & Rice, 1998; Clewell, et al., 1991)

Transposons

Many varieties of transposons have played important roles in the emergence and spread of antimicrobial resistance in enterococci. As more strains become sequenced, it is clear that the enterococcal genome is quite plastic, with mobile elements liberally sprinkled throughout and implicated in a variety of genome rearrangements and transfers. Much of the work to define specific transposons has involved elements that encode resistance to antimicrobial agents, for their obvious clinical importance and for the relative ease of following the movement of such elements. It is now clear, however, that enterococcal transposons confer mobility to a variety of determinants that impact various phenotypes, such as virulence, colonization ability and cell-to-cell communication.

Enterococcal transposons can be broadly classified into three basic categories: composite transposons;, Tn*3*-family transposons, and conjugative transposons (Table 1). Composite transposons are those elements whose mobility is conferred by flanking insertion sequences, or IS elements. In general, the IS elements that form the ends of a composite transposon are identical, and may be oriented as direct or as inverted repeats. Several composite transposons have been described in enterococci, and perhaps the most commonly identified composite transposon are those of the Tn*5281* type (Hong, Hutchings, & Buttner, 2008). Tn*5281* encodes resistance to all aminoglycosides except streptomycin. It consists of the *aac*-6′/*aph*-2′′ bifunctional aminoglycoside-modifying enzyme gene flanked by inverted copies of the insertion sequence IS*256*. As originally described, Tn*5281* has two IS*256* copies on one end and one on the other. In most respects, it is identical to Tn*4001*, a staphylococcal composite transposon in which the bifunctional gene is flanked by single inverted copies of IS*256* (Magnet, et al., 2007). The bifunctional enzyme is responsible for the vast majority of gentamicin resistance observed in enterococci, and it is found almost exclusively in the context of Tn*5281*-like structures.

Tn*1547* is a composite element conferring mobility to the VanB glycopeptide resistance determinant in *E. faecalis* (Rand, Houck, & Silverman, 2007). This 64kb element owes its mobility to flanking copies of IS*16* and an IS*256*-like element. Although Tn*1547* was the first of the VanB glycopeptide resistance elements in enterococci to be

described, subsequent work has shown that the VanB operon is far more frequently associated with Tn*5382*-like elements (Bjørkeng, Rasmussen, Sundsfjord, Sjöberg, Hegstad, & Söderquist, 2011; Dahl, Lundblad, Rokenes, Olsvik, & Sundsfjord, 2000; Lyon, May, & Skurray, 1984).

An interesting example of how IS elements can yield a variety of different mobile elements can be observed with Tn*5385*, a multi-resistance composite element identified in *E. faecalis* (Figure 2). Tn*5385* is a 65kb mobile element whose ends are composed of directly-repeated copies of IS*1216*. Several other putative transposons lie within the larger element, including Tn*4001*, which confers gentamicin resistance, and Tn*5384*, a 26kb element formed by one IS*256* terminus of Tn*4001* and a second IS*256* located 26kb away (Rice L. B., et al., 2009). Between these two IS elements lay determinants for gentamicin, macrolide, and mercury resistance, which makes Tn*5384* a multi-resistance mobile element. Although not conjugative by itself, Tn*5384* is capable of transposing from the *E. faecalis* chromosome to a transferable plasmid. Also included within the larger Tn*5385* are a Tn*916*-like conjugative transposon designated Tn*5381* and an element identical to the staphylococcal β–lactamase transposon Tn*552* (Rice L. B., Carias, Hutton-Thomas, & Rudin, 2007). The mechanisms by which Tn*5385* transfers between enterococcal strains has not been well delineated, but there are data to suggest that its insertion into the recipient chromosome can occur either through homologous recombination across flanking sequences, or through recombination across internal mobile elements and similar structures in the recipient chromosome.

The first Tn*3*-family element described in enterococci was Tn*917*, a 5kb element that confers resistance to macrolides, lincosamides, and streptogramin B through expression of the *ermB* gene (Shaw & Clewell, 1985). Sequence analysis showed that Tn*917* was identical to the macrolide-resistance transposon Tn*551* described in *S. aureus* (Wu, de Lencastre, & Tomasz, 1999). Tn*917* transposes through a replicative mechanism, and resistance expression is repressed by translational attenuation inducible by exposure to erythromycin (but not clindamycin) (Hall, Steed, Arias, Murray, & Rybak, 2012). Neither Tn*917* nor any other Tn*3*-family element encodes transfer functions.

A second Tn*3*-family element that has been identified, albeit rarely, in enterococci is the staphylococcal β–lactamase transposon Tn*552* (Sacco, et al., 2010). Tn*552* expression of β–lactamase is inducible in staphylococci by exposure to penicillin and other related drugs. It is interesting that in most of the β–lactamase-producing *E. faecalis* isolates that have been described, the regulation genes found upstream of the structural β–lactamase gene have been deleted or inactivated, which suggests that the expression of the regulation genes may reduce the fitness of those enterococcal isolates (Ono, Muratani, & Mastumoto, 2005; Rowland & Dyke, 1990; Tomayko, Zscheck, Singh, & Murray, 1996). The consequence of loss of the induction method is that β–lactamase in these strains is expressed constitutively, but at very low levels, resulting in only a modest impact on susceptibility of the isolates. Perhaps the fact that little resistance advantage is achieved explains the rarity with which such strains are isolated. A recent report identified an identical β–lactamase, including the regulatory gene cluster, in eight strains of *E. faecium* (Sarti, Campanile, Sabia, Santagati, Gargiulo, & Stefani, 2012). The impact of this β–lactamase expression on ampicillin susceptibility was marginal, given the higher levels of intrinsic resistance to β–lactams that is characteristic of this species.

The Tn*3*-family transposon that has had the greatest impact on the management of enterococcal infections over the past two decades is Tn*1546*, an 11kb element that encodes the VanA-type glycopeptide resistance operon (Arthur, Molinas, Depardieu, & Courvalin, 1993). As with other resistance genes in Tn*3*-family transposons found in enterococci, the expression of the VanA operon is inducible by exposure; in this case, exposure to glycopeptide antibiotics (Arthur, Molinas, & Courvalin, 1992). The VanA operon is always found in the context of Tn*1546*, although as more clinical isolates are sequenced, increasing numbers of variants (generally created by the insertion of IS elements within parts of the transposon, sometimes with deletion of non-essential segments) are reported. In one recent report, Tn*1546* was modified by insertion of the *fosB* gene, which resulted in an element that confers resistance to both vancomycin and fosfomycin (Quintiliani, Jr. & Courvalin, 1996). Tn*1546*

can be found on both the chromosome and transferable plasmids. Interspecies transfer of these Inc18-type VanA-encoding plasmids has been examined *in vitro*, and the rate of transfer is considerably lower than that seen between enterococcal strains (Werner, et al., 2011). Transfer of vancomycin resistance to *S. aureus* has involved exclusively Tn*1546*-like elements, and in most cases is associated with Inc18-type plasmids (Zhu, et al., 2010).

Conjugative transposons were described at roughly the same time in enterococci and *Streptococcus pneumoniae*. The first enterococcal conjugative transposon described was Tn*916*, which is 18kb in length and confers resistance to tetracycline and minocycline through the *tet(M)* gene (Friedman, Alder, & Silverman, 2006). Its pneumococcal counterpart is Tn*1545* (Courvalin & Carlier, 1987)—and although it was several kb larger than Tn*916* and conferred resistance to macrolides and chloramphenicol in addition to tetracycline and minocycline, Tn*1545* had identical ends and genes that conferred excision and integration capabilities (Clewell, Flannagan, & Jaworski, 1995). Conjugative transposons excise from their point of origin and transfer to their target sites without replication; as a result, their transposition is considered to be conservative. The circular intermediate that forms by excision appears to have heteroduplex DNA at its "joint" region, and the formation of this circle allows transcription to proceed through to the putative conjugation genes (Celli & Trieu-Cuot, 1998). As a result, excision stimulates intercellular transfer. Their host ranges appear to be broad and insertion specificity lax, although they generally appear to target regions that are rich in adenines and thymidines.

Tn*916*-like elements appear to be the primary mechanism by which *tet(M)* genes spread among Gram-positive bacteria. They can transfer to Gram-negative bacteria *in vitro* and their remnants have been found around *tet(M)* genes present in tetracycline-resistant strains of *Neisseria gonorrhoeae* (Swartley, McAllister, Hajjeh, Heinrich, & Stephens, 1993). They encode their own conjugation genes, although they may also transfer between strains incorporated into transferable plasmids. To date, no compelling data exist that conjugative transposons can mobilize other replicons.

The first expansion of the Tn*916*-family transposons came with the description of Tn*5382*, which is an approximately30 kb element that encodes the VanB glycopeptide-resistance operon and has substantial homology with the Tn*916* integrase and excisase genes (Carias, Rudin, Donskey, & Rice, 1998). It also transposes through a circular intermediate. It is able to transfer between enterococcal strains, but in most cases, this transfer appears to occur within larger segments of transferred chromosomal DNA. A likely identical transposon was fully sequenced after the discovery of Tn*5382*, was designated Tn*1549* (Gavaldà, et al., 2007), and is frequently referred to as Tn*5382*/*1549*. Molecular studies of diverse enterococcal strains suggest that Tn*5382*/*1549* is the main structure within which the VanB glycopeptide resistance operon is located (Bjørkeng, Rasmussen, Sundsfjord, Sjöberg, Hegstad, & Söderquist, 2011; Dahl, Lundblad, Rokenes, Olsvik, & Sundsfjord, 2000; Lyon, May, & Skurray, 1984).

With the availability of increasing numbers of fully sequenced genomes, it has become clear that Tn*916*-family transposons are present in many strains and incorporate a variety of different genetic structures. One such transposon, Tn*5386*, is located in the genome of *E. faecium* strain D344R and was shown to interact with a second Tn*916*-like element to yield the deletion of a large segment of chromosome that includes the *pbp5* ampicillin resistance determinant, thereby rendering this *E. faecium* mutant susceptible to ampicillin (Rice L. B., Carias, Marshall, Rudin, & Hutton-Thomas, 2005; Rice, Carias, & Marshall, 1995). Tn*916* itself has been shown to mediate significant changes in the pulsed field patterns of bacterial strains, which suggests that these elements may be major drivers of genomic evolution (Thal, Silverman, Donabedian, & Zervos, 1997). Tn*5386* does not encode tetracycline/minocycline resistance. Instead, what appears to be a bacteriocin immunity operon is located in the place of *tet(M)* . As more genomes sequences are made available for analysis, there is little doubt that the variety of these elements will increase.

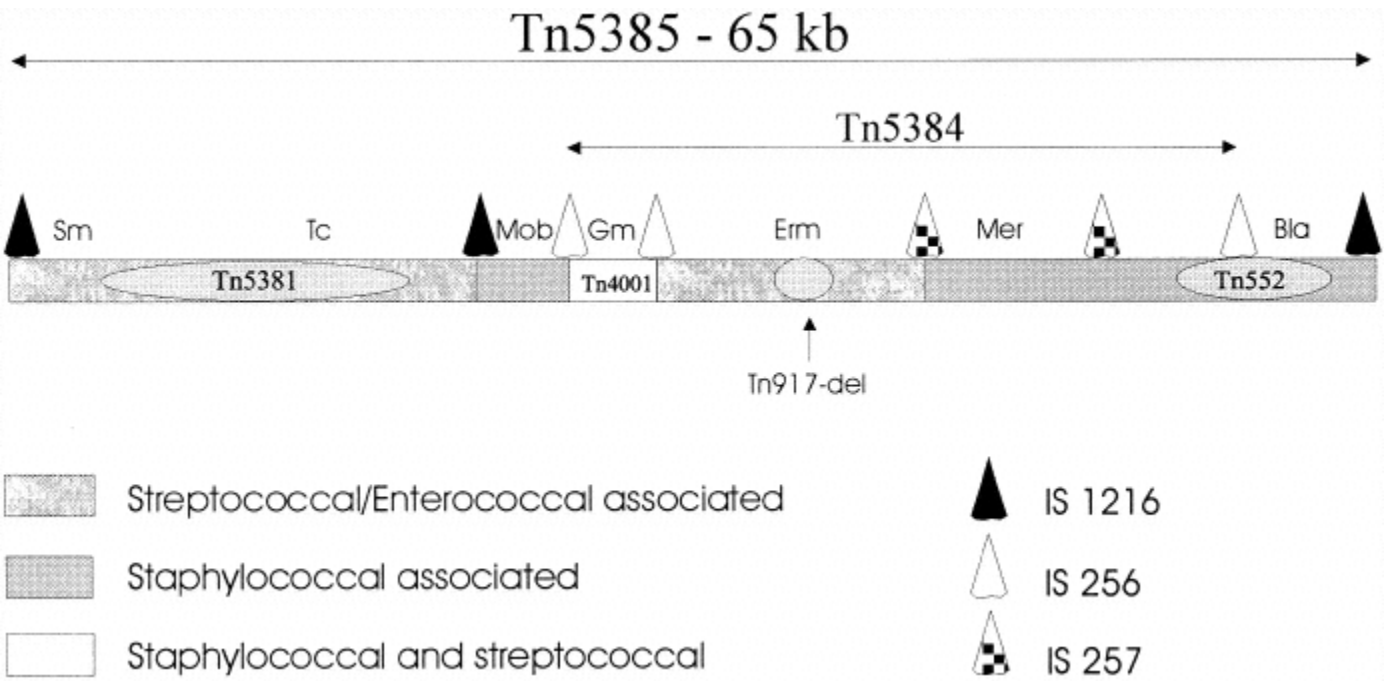


Figure 2. Graphic depiction of the structure of Tn*5385*. The key indicates the presumed origins (based on sequence data reported elsewhere) of the different regions of Tn*5385*. The figures marking the placement of insertion sequences are also indicated in the key. Transposons within the larger element are indicated in various ways, which depend on their degree of overlap. Resistance genes and other important loci are noted above the element. Bla, the β-lactamase gene; Erm, the erythromycin-resistance determinant; Gm, the *aac*-6′-*aph*-2″ bifunctional aminoglycoside-resistance gene; IS, the insertion sequence; Mer, the mercuric chloride-resistance determinant; Mob, the mobilization region, similar to that found on small staphylococcal plasmids; Sm, the *aadE* streptomycin-resistance gene; Tc, the tet(M) tetracycline-minocycline-resistance determinant within Tn*5381*; Tn*917*-del, the region that contains a version of Tn*917* in which the transposition genes have been deleted.

Genomic exchange in *E. faecalis* and *E. faecium*

The emergence and spread of glycopeptide-resistant *E. faecium* has prompted extensive molecular investigations of the strains that both colonize and infect hospitalized patients. Early phenotypic studies suggested differences between strains that colonized patients in Europe but rarely caused infections, and those that caused infections in hospitalized patients in the United States. While both types of strains carried similar determinants that conferred glycopeptide resistance, it was quickly apparent that the infecting strains in the U.S. were far more likely to express high levels of resistance to ampicillin than were the colonizing strains from Europe (Descheemaeker, Chapelle, Devriese, Butaye, Vandamme, & Goossens, 1999). These distinctions led to hypotheses that the strains on the two continents represented two different lineages. The U.S. strains appeared to be a hospital-adapted lineage with higher degrees of resistance to antibiotics, whereas the European strains appeared to be of community origin that were perhaps transferred from the feces of animals that had been given the growth-promoting glycopeptide antibiotic avoparcin. Further work has also demonstrated that the hospital-adapted strains were more likely to contain genes that encode putative virulence determinants such as enterococcal surface protein (*esp*Efm) and hyaluronidase (*hyl*Efm), and that some of these virulence determinants can be encoded on transferable plasmids (Le Breton, Muller, Auffray, & Rincé, 2007; Rice & Marshall, 1992).

It was not especially surprising when transfer of vancomycin-resistance determinants between *E. faecium* strains was demonstrated *in vitro*, since these determinants were known to be encoded on transposons—some of which may be conjugative, but all of which may be incorporated into conjugative plasmids. What was surprising was the demonstration that resistance to ampicillin, which is encoded on the chromosome by the intrinsic *pbp5* gene,

was also transferable at a readily detectable frequency *in vitro* ((Rice L. B., 2005). Exquisite and painstaking work by Manson and colleagues in *E. faecalis* showed that transfers of large regions of the *E. faecalis* V583 genome could be transferred to *E. faecalis* recipients, in the presence of conjugative plasmids (Marshall, Donskey, Hutton-Thomas, Salata, & Rice, 2002). These plasmids contain IS elements identical to those within the enterococcal genome. Cross-over between the IS elements in the plasmids and the chromosome create cointegrates that then use the transfer mechanism of the plasmid to transfer variable-length segments of the donor chromosome. Once these segments are in the recipient cell, these regions can then integrate into the recipient genome, either through homologous recombination or the activity of the transferred IS element. These investigators placed a selectable marker at regions throughout the donor genome and showed that virtually any region could be transferred to the recipient, as long as the pheromone-responsive plasmid was present in the donor. They concluded that these chromosomal mobilizations were the result of recombination between plasmid and chromosomal copies of IS*256*. Similar evidence for IS element-mediated transfer of large genomic resistance segments in *E. faecium* has been observed in one of our laboratories (L.B.R., unpublished).

It now appears that such genomic exchange is common among enterococci isolated in clinical settings. As noted above, it is estimated that as much as 25% of the *E. faecalis* V583 genome has been acquired (Polidori, et al., 2011). Moreover, careful work performed by R. Willems and colleagues has identified a specific loose lineage of *E. faecium*, designated clonal complex 17 (CC17), that is responsible for the vast majority of infection-causing *E. faecium* worldwide (220). This clonal complex first became prevalent in the U.S., while the less resistant and pathogenic strains were becoming common in Europe (67). Prohibition of the use of avoparcin to promote growth in food animals led to reductions in colonization by non-virulent and non-resistant strains, and since that time, CC17 strains have become the predominant disease-causing *E. faecium* strains worldwide (de Regt, et al., 2008). Analysis of *pbp5* genes from CC17 and other strains does not suggest that the entire ampicillin-resistant *E. faecium* outbreak is due to the transfer of single *pbp5* genes among strains, but rather that several different lineages of mutated *pbp5* genes have contributed to the outbreak of ampicillin-resistant, glycopeptide-resistant *E. faecium* (Garnier, Taourit, Glaser, Courvalin, & Galimand, 2000). Recent work also suggests that the CC17 lineage predates the clinical use of antibiotics, suggesting that it is an opportunist that is ideally suited to the antibiotic era, rather than a strain created by selective antibiotic pressure (Galloway-Peña, Rice, & Murray, 2011).

Antibiotics and colonization by multi-resistant *E. faecium*

As noted above, CC17 *E. faecium* strains are ideally suited to proliferate in a world occupied by immunocompromised patients who are given large and prolonged doses of antimicrobial agents. Although they are intrinsically more resistant to ampicillin than their *E. faecalis* counterparts, the emergence of *E. faecium* strains that were highly resistant to ampicillin occurred during the 1980s (Gryczan, Grandi, Hahn, Grandi, & Dubnau, 1980). The scattered reports of the emergence of these strains merited little attention at the time, mostly because *E. faecium* was a relatively minor cause of infection (relative to *E. faecalis*, for instance), and because vancomycin was always available for the treatment of resistant strains. The emergence of glycopeptide resistance in enterococci caught everyone's attention, primarily because there was significant concern that this resistance could transfer to methicillin-resistant *S. aureus*, for which vancomycin was the only reliable therapy at the time. Over time it became apparent that the vast majority of glycopeptide-resistant enterococci were *E. faecium*, both in Europe and in the United States.

With the exception of *E. faecalis* V583, the early glycopeptide-resistant enterococcal strains were isolated and characterized in Europe (Shlaes, Bouvet, Devine, Shales, al-Obeid, & Williamson, 1989; Williamson, Al-Obeid, Shlaes, Goldstein, & Shlaes, 1989). The use of the glycopeptide antibiotic avoparcin as a growth promoter in European food animals suggested that these strains may have arisen in the gastrointestinal tracts of animals, and were transmitted to humans. Early data suggested that a significant percentage of individuals in the European community were colonized with GRE, and that GRE could be isolated from food purchased in supermarkets

(Wegener, Madsen, Nielsen, & Aarestrup, 1997). However, very few GRE infections were reported in European hospitals.

The emergence of GRE in the United States occurred later than its emergence in Europe and had a distinctly different character. U.S. GRE strains became significant causes of infection in hospitalized patients, especially in those that were significantly immunocompromised (Vergis, et al., 2001). Moreover, these strains were far more likely to express high levels of resistance to ampicillin than the strains isolated in Europe. Avoparcin had never been used in U.S. farms and GRE were essentially absent from the feces of U.S. farm animals. Similarly, attempts to isolate GRE from the feces of community dwellers were unsuccessful (Coque, Tomayko, Ricke, Okhyusen, & Murray, 1996). In essence, the European GRE outbreak was a community phenomenon that did not extend significantly into the hospital, and the U.S. outbreak was a hospital phenomenon that did not extend significantly into the community.

Both outbreaks were the results of the application of significant concentrations of glycopeptide antibiotics to the gastrointestinal tract. In Europe, this occurred on farms; in the U.S., it occured in the gastrointestinal tracts of hospitalized patients who were being treated with oral vancomycin for antibiotic-associated diarrhea due to *Clostridium difficile*. These concentrations of glycopeptides eliminated much of the Gram-positive flora of the colon, but left the enterococci, which are tolerant to the bactericidal activity of these agents. Naturally glycopeptide-resistant bacteria then colonized the gut, passing their glycopeptide resistance genes to the resident enterococci.

In animals, this transmission yielded vancomycin-resistant *E. faecium* that otherwise reflected the routine enterococci of the animals, which would be generally susceptible to ampicillin and not well suited for pathogenicity. In humans, transmission occurred into strains that were already hospital-adapted; in other words, strains that were more resistant to antibiotics and those that were more likely to express virulence determinants that promote infection. Recent data suggest that CC17 strains, which are well suited for survival and pathogenicity in the hospital, have been present for a long period of time (Galloway-Peña, Rice, & Murray, 2011). Prior to the emergence of GRE, these strains became the predominant hospital flora when extended-spectrum cephalosporin use became widespread, because extended-spectrum cephalosporins are potent selectors of ampicillin-resistant *E. faecium* colonization of the gastrointestinal tract (Chirugi, Oster, Goldber, & McCabe, 1992). When the vancomycin-resistance determinants became available, they were transferred into these strains. In Europe, it appears that the prevalence of the non-pathogenic strains from animals forestalled the emergence of glycopeptides-resistant CC17 strains. Political pressure led to the discontinuation of glycopeptide use in animals in the 1990s. Since that time, the prevalence of GRE in farms animals and in the community has decreased. Unfortunately, many European countries now have increasing reports of nosocomial outbreaks of infection and colonization, caused by CC17 strains (Bourdon, et al., 2011).

An understanding of the impact of antibiotic administration on colonization by GRE is important when evaluating strategies to try to reduce GRE gastrointestinal colonization in the clinical setting. For example, shortly after the likely association between oral vancomycin therapy and GRE colonization was recognized, recommendations were proposed that *C. difficile*-associated diarrhea should be treated with metronidazole rather than vancomycin (Cohen, et al., 2010). Before long, however, it was recognized that exposure to metronidazole was also associated with an increased risk of GRE colonization (Cervera, et al., 2011). Animal studies confirmed that while anti-anaerobic antibiotics, such as metronidazole, promoted the persistence of high levels of GRE gastrointestinal colonization, antibiotics that lacked anaerobic activity did not (Donskey, Hanrahan, Hutton, & Rice, 1999). Donskey and colleagues (Donskey, et al., 2000) extended these studies to humans, and showed that fecal output of VRE significantly increased after colonized patients were exposed to anti-anaerobic antibiotics.

It was also surprising to many that association of GRE colonization with intravenous vancomycin exposure was only inconsistently observed in clinical studies, and that association with exposure to extended-spectrum

cephalosporins was much more consistently found (Carmeli, Eliopoulos, & Samore, 2002). However, intravenous vancomycin is not secreted into the gastrointestinal tract in detectable concentration, at least in the first five days of therapy (Currie & Lemos-Filho, 2004). On the other hand, some extended-spectrum cephalosporins are secreted into the gastrointestinal tract in large concentrations (especially ceftriaxone). A series of animal studies convincingly documented that the selection of GRE colonization by ceftriaxone is dependent on the expression by the GRE of high levels of ampicillin resistance, which translate to very high levels (>5,000 µg/ml) of ceftriaxone resistance (Rice, Lakticová, Carias, Rudin, Hutton, & Marshall, 2009). Since within a short period of time more than 90% of GRE isolated in the U.S. were ampicillin-resistant *E. faecium*, the connection between extended-spectrum cephalosporins and GRE is understandable. It is interesting to note that these animal studies also suggested that piperacillin-tazobactam can promote the persistence of high levels of GRE colonization by virtue of being a potent anti-anaerobic agent, but can prevent the establishment of GRE colonization, through its inhibitory effect on growth of GRE in the upper gastrointestinal tract (Donskey, Hanrahan, Hutton, & Rice, 1999). Consistent with these findings, piperacillin-tazobactam has been only inconsistently associated with GRE colonization in clinical studies.

Enterococcal antimicrobial resistance and the use of antibiotics in food animals

As previously noted, there are convincing data to suggest that the emergence of VRE in the European population is tied to the use of the glycopeptide antibiotic avoparcin in food animals. The use of growth-promoting or therapeutic antibiotics in animals has been associated with a variety of resistance determinants found in human isolates of the same species. Specifically, enterococcal resistance to quinupristin-dalfopristin has been tied to the use of virginiamycin in food animals (Welton, et al., 1998). Whether the specific strains that emanate from animals are likely to cause disease in humans can be argued, but should not be used in an argument to promote the continued use of antibiotics in animals for the purpose of increased growth. Recent data are clear that the intra-species transfer of enterococcal resistance determinants is far more common than inter-species transfer (Dahl, et al., 2007), and that the rates of transfer *in vivo* exceed those that we are able to measure *in vitro*. As such, any practices that encourage the acquisition of important resistance determinants by any enterococcal strains should be avoided.

Enterococcal Infection: Strategies for, and Consequences of, Antibiotic Treatment

Treatment of enterococcal infections has become one of the most challenging issues facing clinicians in the 21st century. The increased prevalence of strains that are resistant to almost all antibiotics with *in vitro* bactericidal activity against enterococci is a worrisome trend. Indeed, among the most common enterococcal species isolated from critically ill patients in the USA, *Enterococcus faecium* has become one of the most predominant, becoming almost as often isolated from clinical samples at some hospitals as *Enterococcus faecalis* (Hodel-Christian & Murray, 1991; Mir, Asong, Li, Cardot, Boons, & Husson, 2011). This trend in the epidemiology of enterococcal infections has important clinical consequences, since resistance to antibiotics such as ampicillin and vancomycin (which used to be the cornerstone antibiotics for the treatment of enterococcal infections) is the "norm" in modern-day hospital-associated isolates of *E. faecium*. Conversely, resistance to ampicillin is rarely seen in *E. faecalis*, and vancomycin resistance is much less frequent in this species. Furthermore, the infectious Disease Society of America (IDSA) has included *E. faecium* among the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, and *Enterobacter* spp.) for which new therapies are urgently needed (Arias & Murray, 2012; Rice, et al., 2004).

One of the main challenges in the treatment of severe enterococcal infections is that these organisms are either tolerant or intrinsically resistant to a variety of antimicrobial agents. For example, compared to streptococci,

higher concentrations of penicillin (10 to >100 times) than those used against streptococci are needed to kill enterococci *in vitro* (Murray, 2000). Moreover, enterococci are often tolerant to β-lactam antibiotics with minimal bactericidal concentrations (MBC) of penicillin and ampicillin that exceed ≥32 times the minimal inhibitory concentration (MIC) of the corresponding β-lactam compound. Also, enterococci often possess genes that make them intrinsically resistant to compounds used in clinical practice. For example, *E. faecium* carries an acetylase enzyme (AAC(6′)) that produces higher MICs to several aminoglycosides (such as tobramycin and amikacin), which results in a loss of synergistic effect with cell-wall agents (Arias, Contreras, & Murray, 2008). Similarly, most *E. faecalis* contain a gene (designated *lsa*) that encodes an ATP-binding protein which confers intrinsic resistance to quinupristin/dalforistin (Q/D) (Singh, Weinstock, & Murray, 2002). These therapeutic issues have had an important impact since the early days of the antibiotic era (1940s); indeed, cure rates in the treatment of enterococcal endocarditis with penicillin monotherapy were disappointing (~60%) when compared to those for streptococcal endocarditis (Arias, Contreras, & Murray, 2010; Murray, 2000), and only the addition of an aminoglycoside increased the number of favorable outcomes.

We will focus the discussion in this section on issues related to the treatment of severe enterococcal infections (namely, bacteremia and endocarditis) in both susceptible and multidrug-resistant (MDR) enterococci. Additionally, we will include comments related to the influence of antibiotic therapy in the selection of drug resistance, and will discuss the changes in the gastrointestinal (GI) tract caused by the use of antibiotics that promote the growth and dissemination of MDR enterococci.

Treatment of enterococcal infections: susceptible vs. resistant enterococci

The majority of enterococcal infections are described in critically ill patients for whom treatment is usually required. The clinical approach to the treatment of susceptible versus resistant enterocoal infections differs significantly. For practical purposes, the majority of modern-day "susceptible" enterococcal infections are caused by isolates of *E. faecalis* that lack resistance to ampicillin (or penicillin) and vancomycin, and do not exhibit high-level resistance (HLR) to aminoglycosides. Nonetheless, although ampicillin resistance continues to be uncommon in clinical isolates of *E. faecalis*, high-level resistance (HLR) to aminoglycosides has become more common, and vancomycin resistance appears to be increasing. On the other hand, most MDR enterococcal infections are caused by *E. faecium*, in which both ampicillin and vancomycin have become obsolete, and the isolates often exhibit HLR to aminoglycosides. Thus, we will divide this section in three parts: *i*) treatment of *Enterococus faecalis* infections that are susceptible to ampicillin and vancomycin, and do not exhibit HLR to aminoglycosides; *ii*) treatment of *E. faecalis* infections that are susceptible to ampicillin and vancomycin but exhibit HLR to aminoglycosides; and *iii*) treatment of MDR *E. faecium*.

Treatment of ampicillin and vancomycin-susceptible *Enterococus faecalis* without HLR to aminoglycosides

The cornerstone for treating susceptible enterococcal infections is the β-lactam antibiotics, and vancomycin is usually reserved for cases in which ampicillin cannot be used, due to a β-lactam allergy. Among the β-lactams, the compounds with the best *in vitro* activity include the amino-penicillins (such as ampicillin) and ureidopenicillins (such as piperacillin), followed by penicillin G and carbapenems (imipenem). However, as previously mentioned, the use of β-lactam monotherapy is often non-bactericidal for enterococci, and was associated with poor outcomes in endovascular infections. Of note, a bactericidal regimen is of paramount importance to the treatment of deep-seated infections such as endocarditis. Pioneer studies performed in the 1970s (Moellering, Jr. & Weinberg, 1971; Zimmermann, Moellering, Jr., & Weinberg, 1971) provided compelling evidence that the association of a cell-wall agent, such as a β-lactam (or a glycopeptide, such as vancomycin) plus an aminoglycoside produced a synergistic bactericidal effect against enterococci. Synergism is defined *in vitro* as a decrease of >2 log10 in bacterial counts (CFU/ml) by 24 hours, as compared to the cell wall agent alone (Moellering, Jr., Linden, Reinhardt, Blumberg, Bompart, & Talbot, 1999), or a 99.9% reduction in colony counts

from the starting inoculum when the combination of a β-lactam (or glycopeptide) and an aminoglycoside is used. Additionally, the concentration of the aminoglycoside should not have any effect on the growth curve of the tested microorganism (Murray, 2000). The basis for such a synergistic effect appears to be related to the increase in the uptake of the aminoglycoside molecule by the bacterium when the cell wall is altered (Arias, Contreras, & Murray, 2008).

Gentamicin and streptomycin are the two main aminoglycosides used in clinical practice to achieve bactericidal therapy, and clinical experience supports the use of the combination of ampicillin (or penicillin) and either of these two aminoglycosides as the first line of therapy for severe enterococcal infections that are susceptible to both classes of antibiotics. It is of paramount importance that the clinical microbiology laboratory tests isolates for the presence of HLR to aminoglycosides, since the presence of HLR abolishes the synergistic effect. HLR is defined in the clinical laboratory by the presence of at least one colony-forming unit at concentrations of 2000 mg/L and 500 mg/L of streptomycin and gentamicin, respectively, on brain heart infusion agar (BHI), or evidence of growth at a concentration of 1000 mg/L of streptomycin when using BHI broth (Mir, Asong, Li, Cardot, Boons, & Husson, 2011). It is also important to note that the use of aminoglycosides other than gentamicin or streptomycin is not recommended for the treatment of severe *E. faecalis* infections where synergistic bactericidal therapy is necessary, since many enterococcal isolates may harbor the *aph(3′)-IIIa* gene, which confers HLR to kanamycin and resistance to the synergistic effect of amikacin (Mir, Asong, Li, Cardot, Boons, & Husson, 2011). Moreover, testing for the presence of resistance or a lack of synergism to aminoglycosides other than gentamicin or streptomycin has not been standardized for routine use in the clinical laboratory.

Some clinicians have advocated the use of continuous infusions of penicillin for the treatment of enterococcal endocarditis (Vogler, Dorney, & Bridges, 1962) in order to improve the time above MIC (*T*>MIC) parameter that is crucial for β-lactam-related killing. However, animal models of enterococcal endocarditis have shown conflicting results. Using a rat endocarditis model, Thauvin et al. (Thauvin, Eliopoulos, Willey, Wennersten, & Zervos, 1987) showed that continuous infusion of ampicillin was superior than intermittent intramuscular injection in reducing bacterial titers in cardiac vegetations. Conversely, Hellinger et al. (Hellinger, Rouse, Rabadan, Henry, Steckelberg, & Wilson, 1992) did not find any difference in efficacy between the administration of ampicillin by continuous infusion or intermittent administration (both by intravenous route and in combination with gentamicin) in an experimental model of rabbit endocarditis. Prospective clinical data comparing the two regimens in humans are lacking, and as a result, it is difficult to make any recommendations for the use of one therapeutic strategy above the other.

In rare cases of *E. faecalis* infections, the presence of β–lactamase in these isolates may compromise the clinical effectiveness of ampicillin or penicillin (Arias, Singh, Panesso, & Murray, 2007; Norris, Reilly, Edelstein, Brennan, & Schuster, 1995). The Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2012) recommends testing for penicillinase in specific clinical settings, such as endocarditis. These isolates should not cause major therapeutic dilemmas, since the ampicillin-sulbactam combination should be effective against these penicillinase-producing strains (Table 2).

Table 2. Options for the treatment of severe *E. faecalis* infections

Ampicillin and vancomycin-susceptible *E. faecalis* without HLR to aminoglycosides
Ampicillin (or penicillin) plus aminoglycosides (gentamicin or streptomycin) In the case of a β-lactam allergy, vancomycin is the drug of choice

Ampicillin and vancomycin-susceptible *E. faecalis* with HLR to aminoglycosides
Ampicillin plus ceftriaxone Ampicillin plus imipenem plus vancomycin Ampicillin plus fluoroquinolones[1] Ampicillin plus high-dose daptomycin[2]
Penicillinase-producing *E. faecalis* (rare)
Ampicillin-sulbactam plus aminoglycosides (gentamicin or streptomycin)
Vancomycin-resistant *E. faecalis*
They are usually susceptible to ampicillin, and ampicillin-based regimens are preferred

Treatment of ampicillin and vancomycin-susceptible *E. faecalis* that exhibit HLR to aminoglycosides

One of the important clinical challenges in the treatment of E. faecalis endovascular infections is the emergence of HLR to gentamicin, which is usually mediated by the bifunctional aminoglycoside modifying enzyme AAC(6′)-Ie-APH (2′′)-Ia (Chow, 2000), as well as HLR to streptomycin, which is associated with ribosomal mutations and/or the presence of an streptomycin-nucleotidyltransferase enzyme (9). In some regions of the world, the prevalence of E. faecalis with HLR to aminoglycoside appears to be increasing, and represents up to 60% of the isolates (Patel, et al., 2001). HLR to gentamicin or to streptomycin abolish the synergistic bactericidal effect of the cell-wall agent/aminoglycoside combination in most E. faecalis isolates and represent a serious issue in the treatment of IE. Another important consideration is that, due to the toxicity profile of aminoglycosides, clinicians are sometimes reluctant to use the aminoglycoside class of antibiotics in critically ill patients who may have compromised renal function. Therefore, alternatives to the aminoglycosides are an important clinical necessity in patients in whom these compounds cannot be used due to resistance or toxicity.

Clinical and animal studies have shown that the combination of cephalosporins (such as ceftriaxone or cefotaxime) with an amino-penicillin (such as ampicillin or amoxicillin) is synergistic and bactericidal against strains of E. faecalis that exhibit HLR but are fully susceptible to ampicillin (Mainardi, et al., 2007; Tascini, Doria, Leonildi, Martinelli, & Menichetti, 2004). The basis for this effect is thought to be the result of the differential saturation of the E. faecalis penicillin-binding proteins (PBPs) by ampicillin and cefotaxime (or ceftriaxone). The amino-penicillin may partially saturate PBPs 4 and 5, but not PBPs 2 and 3; the latter PBPs may still participate in cell-wall synthesis. In the presence of the cephalosporin/amino-penicillin combination, complete saturation of PBPs 2 and 3 may be achieved, which results in a bactericidal synergistic effect (Mainardi, et al., 2007). Clinical support for the use of the combination of ampicillin (2 g every 12 h) plus ceftriaxone (2 g every 4 h) was provided by a prospective, open-label, non-randomized trial in 13 Spanish hospitals. The study included 43 patients (21 and 22 patients with E. faecalis isolates that exhibited HLR to aminoglycoside and non-HLR, respectively). The rate of clinical cure for isolates with HLR to aminoglycosides and non-HLR was 71.4% and 72.7%, respectively, at the end of treatment (six weeks), and 71.4% and 63.6% at three months, respectively. However, only 13 patients completed the protocol, with an overall clinical cure rate at three months of 67.4%, although microbiological cure was 100%. The data are promising and the combination may be an interesting option for E. faecalis infective endocarditis (IE) in which aminoglycosides cannot be used (Goffin & Ghuysen, 1998) (Table 2). More recently, an observational, nonrandomized, comparative multicenter cohort study at 17 Spanish and 1 Italian hospitals provided robust clinical evidence indicating that the combination of ceftriaxone plus ampicillin was as effective as the ampicillin-aminoglycoside combination for the treatment of E. faecalis with less toxicity (Fines, Perichon, Reynolds, Sahm, & Courvalin, 1999).

In Japan, the aminoglycoside arbekacin, which is more stable than gentamicin to the action of the AAC(6′)-Ie-APH (2′′)-Ia enzyme, is available for the treatment of enterococcal infections. Interestingly, in vitro studies have

found that arbekacin produced synergism when combined with ampicillin in 40% of enterococci that possessed the AAC(6′)-Ie-APH (2′′)-Ia enzyme (Kinnebrew, Ubeda, Zenewicz, Smith, Flavell, & Pamer, 2010). Moreover, in vivo studies in experimental endocarditis (rabbits) showed that the combination of ampicillin plus arbekacin was able to achieve a more statistically significant decrease in bacterial counts from vegetations, as compared to ampicillin plus gentamicin (Kak, You, Zervos, Kariyama, Kumon, & Chow, 2000), which suggests a possible role of arbekacin in the treatment of certain isolates that exhibit HLR to gentamicin, although prospective, comparative clinical data in humans are not available.

Other regimens that have been used successfully in sporadic cases of infective endocarditis or bacteremia caused by E. faecalis with HLR to aminoglycosides include the combination of ampicillin plus imipenem plus vancomycin (Antony, Ladner, Stratton, Raudales, & Dummer, 1997), and ampicillin plus ofloxacin (Tripodi, Locatelli, Adinolfi, Andreana, & Utili, 1998), and daptomycin plus ampicillin. Additionally, a new generation of cephalosporins with good in vitro activity against some strains of E. faecalis (but not E. faecium) has recently been made available. Ceftobiprole, which is only available in Canada and Switzerland, and ceftaroline (available in the USA) are members of this new class of compounds. The available data suggest that ceftobiprole may have some potential in the treatment of E. faecalis infections. Indeed, ceftobiprole has been shown to exhibit potent in vitro synergistic bactericidal activity against E. faecalis, including against strains that produce β-lactamase and carry a vancomycin resistance gene cluster (Arias, Singh, Panesso, & Murray, 2007). Moreover, ceftobiprole showed potent in vivo activity against E. faecalis in a mouse model of peritonitis (Arias, Singh, Panesso, & Murray, 2007) and urinary tract infection (Singh & Murray, 2012). Further clinical data are needed in order to evaluate the specific role of ceftobiprole in the treatment of E. faecalis infections.

Treatment of multidrug-resistant *E. faecium*

As previously mentioned, the relatively recent rise in frequency of nosocomial infections caused by multidrug-resistant *E. faecium* is one of the most important challenges in clinical settings, since reliable therapies for severe, deep-seated infections caused by these MDR strains are not available. The majority of current *E. faecium* isolates recovered from hospitals across the USA are resistant to ampicillin. Moreover, the ampicillin MICs of nosocomial isolates of *E. faecium* that are currently isolated from hospitals in the USA are much higher than those that were isolated 20 years ago (Garnier, Taourit, Glaser, Courvalin, & Galimand, 2000). This observation has important clinical consequences, since it has been postulated that *E. faecium* isolates with ampicillin MICs $\leq$ 64 μg/ml could potentially be treated with high doses of ampicillin (up to 30 g/day) combined with an aminoglycoside (if HLR is not present) (Murray, Mederski-Samoraj, Foster, Brunton, & Harford, 1986) (Table 3), since concentrations of ampicillin in serum between 100 to 150 mg/L can be readily obtained with a high-dose regimen. Moreover, for an *E. faecium* isolate with an ampicillin MIC of 16 μg/mL, the combination of ampicillin with imipenem (MIC of 32 μg/ml) was superior in reducing bacterial counts from vegetations, as compared with the most active single agent in experimental endocarditis (Brandt, Rouse, Laue, Stratton, Wilson, & Steckelberg, 1996). Unfortunately, *E. faecium* isolates with ampicillin MICs $\leq$ 64 μg/ml are now rare in clinical settings. Furthermore, vancomycin, which used to be the most reliable alternative for the treatment of ampicillin-resistant *E. faecium* isolates, has now become obsolete in a high proportion of infections, particularly in the USA, where more than 80% of isolates are resistant to glycopeptides. Similarly, a high percentage of these isolates also exhibit HLR to both gentamicin and streptomycin, reducing even further the possibilities of obtaining synergistic bactericidal therapy.

Only two compounds are currently approved by the Food and Drug administration for the treatment of vancomycin-resistant *E. faecium* infections: linezolid and quinupristin-dalfopristin (Q/D), and both compounds are recommended by the American Heart Association (AHA) for the treatment of MDR *E. faecium* IE. The clinical experience with both compounds is limited since controlled, prospective, randomized clinical trials are not available. Q/D is a mixture of the streptogramin antibiotics quinupristin (streptogramin B) and dalfopristin (streptogramin A) that inhibit protein synthesis after their interaction with the 50S ribosomal subunit. As

mentioned above, most *E. faecalis* are intrinsically resistant to Q/D—but the antibiotic does have potent *in vitro* bactericidal activity against *E. faecium*. The clinical efficacy of Q/D for the treatment of severe infections caused by MDR *E. faecium* was assessed in two multicentric, prospective, non-comparative studies (Lobritz, Hutton-Thomas, Marshall, & Rice, 2003; Moellering, Jr. & Weinberg, 1971). The overall success rates (both clinical and bacteriological) in both studies were ca. 65%. Of note, Q/D has some important side effects, such as phlebitis and myalgias/arthralgias, which have led to treatment interruptions (Arias, Contreras, & Murray, 2008).

A retrospective analysis of 113 patients with VRE bacteremia treated with Q/D found an attributable mortality rate (related to the infection) of 50%, which was significantly higher than the rate observed in patients treated with linezolid (p=0.002) (Erlandson, Sun, Iwen, & Rupp, 2008). Moreover, the use of Q/D monotherapy has been associated with several reports of clinical failure, which has led to the suggestion that Q/D may be used as part of a combination regimen against isolates of MDR *E. faecium* (Table 3). Indeed, the association of Q/D plus doxycycline and rifampin cleared the bloodstream of a patient with VRE endocarditis who had failed Q/D monotherapy (Mave, Garcia-Diaz, Islam, & Hasbun, 2009). Similarly, the association of Q/D plus ampicillin was successfully used in the treatment of a patient with recurrent IE endocarditis due to *E. faecium* (ampicillin MIC >16 μg/ml) after clinical failure with linezolid monotherapy (Bethea, Walko, & Targos, 2004). Thus, the limited clinical data suggests that Q/D may be used as part of a combination therapy when treating severe *E. faecium* infections. (Table 3)

Linezolid is a synthetic oxazolidinone with bacteriostatic activity against enterococci (all species) whose mechanism of action is thought to involve interactions with the A site of bacterial ribosomes that inhibit protein synthesis. As with Q/D, this compound is also FDA-approved for the treatment of VRE infections (both *E. faecalis* and *E. faecium*) and is also recommended by the AHA for IE caused by vancomycin-resistant *E. faecium* (Baddour, et al., 2005). Clinical support for the use of linezolid for severe VRE infections is derived mostly from retrospective studies, case reports, and a small meta-analysis. In a comparative study using linezolid for compassionate indications in VRE infections, favorable clinical and microbiological outcomes were 78% and 85% in the intention to treat population, respectively. However, in the subgroup of patients with VRE IE, microbiological eradication was obtained only in 63% of the patients, whereas 76% achieved a clinical cure (Birmingham, Rayner, Meagher, Flavin, Batts, & Schentag, 2003). Another study which included patients with solid organ transplants and VRE bacteremia reported a clinical success rate with linezolid of only 67% (El-Khoury & Fishman, 2003).

Three retrospective studies have attempted to compare the clinical outcomes of linezolid vs daptomycin in the treatment of bacteremia caused by VRE. The first one by Mave et al. (Mave, Garcia-Diaz, Islam, & Hasbun, 2009) analyzed the clinical outcomes of 68 patients treated with linezolid. Overall mortality was ca. 21% with a median duration of bacteremia of two days and a relapse rate of 2.9%. The overall microbiological eradication rate was 88.2%. The second study included 34 patients treated with linezolid, in which the mortality rate was 29.4%. IE treated with linezolid was associated with a prolonged duration of blood cultures (Crank, et al., 2010). A logistic regression analysis performed in this study suggested that previous linezolid treatment was associated with increased mortality (Odds ratio [OR] 6.63 p=0.031). The third and largest study included 138 patients with VRE bacteremia who were treated with linezolid (Twilla, Finch, Usery, Gelfand, Hudson, & Broyles, 2012). The rates of clinical and microbiological cures were 74% and 94%, respectively, with a rate of recurrence of 3%. The overall mortality in the linezolid arm was ca. 18%. Additionally, a meta-analysis attempted to examine the efficacy of linezolid in the treatment of VRE endocarditis. From six cases reported in this meta-analysis, four were *E. faecium*, and clinical cure with linezolid was achieved in all of them (Falagas, Manta, Ntziora, & Vardakas, 2006). Conversely, clinical failures when using linezolid monotherapy have been reported in several cases of VRE infections (Berdal & Eskesen, 2008; Webster, Griffiths, & Bowler, 2009), including some cases of endocarditis and central nervous system infections. Therefore, with the available clinical data, linezolid appears to be a reasonable option for the treatment of VRE infections; however, its bacteriostatic activity may preclude the routine use of linezolid in endovascular infections. If this is the case, linezolid should be reserved as an alternate

option when other bactericidal therapies are unavailable. Furthermore, a worrisome recent report of transferable plasmid-mediated linezolid resistance in enterococci mediated by the methyl-transferase Cfr (Diaz, Kiratisin, Mendes, Panesso, Singh, & Arias, 2012) raises concern about the possible emergence and rapid dissemination of linezolid resistance in enterococci. Linezolid may be an attractive option for the treatment of central nervous system infections caused by MDR *E. faecium,* due to the favorable pharmacokinetics of the drug in the cerebrospinal fluid and CNS tissues.

Daptomycin is a lipopeptide antibiotic with bactericidal activity against enterococci, including VRE (both *E. faecium* and *E. faecalis*), which makes it an attractive compound for the treatment of MDR *E. faecium* infections. Daptomycin is FDA-approved for the treatment of vancomycin-susceptible enterococcal skin and soft tissue infections, but has no indication for any VRE infection or for *E. faecium*. However, clinicians often use this compound in the treatment of severe VRE infections, due to a lack of other bactericidal options. As we have seen with other compounds, the clinical data that supports the use of daptomcyin for VRE infections are composed mostly of retrospective studies and case reports.

Mohr et al. (Mohr, Friedrich, Yankelev, & Lamp, 2009) retrospectively analyzed 159 patients with enterococcal bacteremia (120 of those patients with *E. faecium*) who received treatment with daptomycin. Clinical success was observed in 104 patients (87%), with adverse events possibly related to daptomycin observed in 9.5%, eight of which were considered serious. Apart from bacteremia, daptomycin has been used in the treatment of several other enterococcal infections, including endocarditis, skin and soft tissue infections, bone and joint infections and urinary tract infections (Cantón, Ruiz-Garbajosa, Chaves, & Johnson, 2010). In IE, which is an infection where bactericidal therapy is necessary for optimal outcomes, the European Cubicin Outcomes Registry and Experience (EU-CORE) reported 22 cases of enterococcal endocarditis (18 of these isolates were *E. faecalis* and 4 were *E. faecium*) in which the overall clinical cure was 73% (Cervera, et al., 2011) but robust clinical data to support the use of daptomycin in enterococcal IE are still lacking. As mentioned above, three studies have retrospectively compared the clinical outcomes of patients with enterococcal bacteremia using linezolid vs daptomycin (Crank, et al., 2010; Diaz, Kiratisin, Mendes, Panesso, Singh, & Arias, 2012; Murray, Mederski-Samoraj, Foster, Brunton, & Harford, 1986). In all three studies, no statistical significant difference in mortality was found between the two compounds (mortality in patients treated with daptomycin ranged from 24% to 46%), although a trend towards increased mortality was observed in one of these comparative studies. Patients in the daptomycin arm appeared to be sicker, making it difficult to extrapolate the data from this study to clinical practice.

An important issue in the treatment of deep-seated enterococcal infections during daptomycin monotherapy is the report of clinical failures and emergence of resistance during therapy (mostly reported in bacteremia and endocarditis) (Arias, et al., 2011). In the majority of these cases, a lack of a clinical cure has been correlated with the use of dose schemes that were approved for the treatment of soft tissue infections (only with vancomycin-susceptible enterococci) and *S. aureus* bacteremia (4 and 6 mg/kg, respectively). Indeed, the MIC breakpoint for enterococci is four times higher than that of staphylococci (4 ug/ml versus 1 ug/ml) (Clinical and Laboratory Standards Institute, 2012), which leads to the suggestion that higher doses are needed for the treatment of enterococcal infections. Thus, doses that range from 8 to 14 mg/kg have been used in several case reports of difficult-to-treat and recurrent enterococcal infections (Mir, Asong, Li, Cardot, Boons, & Husson, 2011). A recent work, which uses an *in vitro* model of simulated endocardial vegetations, suggested that higher doses of this compound achieved the best bactericidal activity and prevented the emergence of resistance (Hancock & Perego, 2002). As previously mentioned for other compounds, prospective clinical trials that have evaluated the efficacy and safety of higher doses of daptomycin are lacking, but the available clinical evidence suggest that doses of 8-10 mg/kg or higher should be strongly considered in the treatment of severe enterococcal bacteremia and endocarditis.

Some of the disappointing outcomes observed with daptomycin monotherapy in the treatment of *E. faecium* infections have been overcome by the addition of compounds with *in vitro* activity against the infecting strains. For example, failure of daptomycin in the treatment of two patients with *E. faecium* endocarditis was overcome by the addition of high-dose ampicillin plus gentamicin, and gentamicin plus rifampin, respectively (Arias, et al., 2007; Stevens & Edmond, 2005). Moreover, three additional patients with recurrent *E. faecium* IE were successfully treated with a combination of daptomycin plus tigecycline (Jawetz & Sonne, 1966; Prystowsky, et al., 2001; Schutt & Bohm, 2009). Tigecycline is a bacteriostatic compound derived from minocycline (tetracycline class of antibiotics) that is approved by the FDA for the treatment of soft tissue infections and community-associated pneumonia caused by susceptible organisms. This antibiotic has good *in vitro* activity against enterococci, concentrates well in intra-abdominal tissues, and readily penetrates vegetations. However, the blood levels achieved by tigecycline are low, making the use of this antibiotic as monotherapy less attractive for enterococcal bloodstream infections. Nonetheless, the combination of tigecycline with daptomycin may be an interesting regimen for the treatment of MDR *E. faecium* IE and difficult-to-treat bacteremias, although more clinical data are needed in order to clarify the role of this combination in the treatment of these infections.

Another intriguing combination used for the treatment of MDR *E. faecium* IE is daptomycin plus ampicillin in an *E. faecium* isolate that exhibited high MICs to ampicillin. Indeed, a recent report by Sakoulas et al. (Sakoulas, et al., 2012) describes a patient with *E. faecium* endocarditis who failed daptomycin monotherapy, but whose bloodstream infection was cleared by adding ampicillin (the isolate had an ampicillin MIC of > 128 μg/ml). Ampicillin appears to enhance the killing of daptomycin in these isolates by mechanisms that are unclear, but seem to be related to the enhanced binding of the lipopetide to the cell membrane. Further clinical data on the use of this combination are needed.

Chloramphenicol is an antibiotic that has been used in the treatment of severe infections caused by VRE (mostly *E. faecium*), since this compound retains *in vitro* activity against most isolates. Chloramphenicol was used in 14 patients with VRE bloodstream infections, achieving 73% of microbiological eradication, but only 53% of favorable clinical response (Okamoto, Okubo, & Inoue, 1996). Another study retrospectively evaluated the clinical response of chloramphenicol treatment for VRE bacteremia in 51 patients (Laverde Gomez, et al., 2011) with rates of microbiological eradication and clinical cure of 79% and 61%, respectively. Moreover, chloramphenicol has been used as an anti-enterococcal agent in cases of CNS infections caused by enterococci (ventriculitis and meningitis) (Heaton & Handwerger, 1995; Scapellato, Ormazabal, Scapellato, & Bottaro, 2005), and also in IE (in combination) (Safdar, Bryan, Stinson, & Saunders, 2002). One of the main concerns regarding the use of chloramphenicol is its inherent bone marrow toxicity, and as a result, the limited clinical data suggest that this compound may be used in "desperate" cases when other therapies or combinations have failed.

Finally, oritavancin is an investigational glycopeptide antibiotic with *in vitro* activity against vancomycin-susceptible enterococci and VRE, including *E. faecium* with the VanA phenotype. *In vivo* studies using animal models of endocarditis (Linden, et al., 2001; Saleh-Mghir, et al., 1999) have shown that oritavancin was able to reduce the number of bacterial colony-forming units of VRE infecting vegetations, as compared to untreated controls and vancomycin, although it did not sterilize the vegetations. Moreover, combination of oritavancin plus gentamicin produced a significant reduction in bacterial counts from vegetations, as compared to untreated controls and prevented emergence of oritavancin-resistant mutants which were readily selected with the use of oritavancin monotherapy. Thus, this compound may offer promise for the treatment of VRE, particularly when used in combination with other agents in the treatment of severe VRE infections (Arias, Mendes, Stilwell, Jones, & Murray, 2012).

Table 3: Possible alternatives for the treatment of severely vancomycin-resistant *E. faecium* infections

E. faecium with ampicillin MIC ≤ 64 µg/ml
No HLR to aminoglycosides
High-dose ampicillin plus aminoglycosides (gentamicin or streptomycin)
HLR to aminoglycosides
HD ampicillin[1] plus daptomycin[2]
HD ampicillin plus Q/D
HD ampicillin plus imipenem[3]
E. faecium with ampicillin MIC > 64 µg/ml
No HLR to aminoglycosides
Daptomycin[2] plus an aminoglycoside + another active agent[4]
Q/D plus + another active agent[4]
Linezolid + another active agent[4]
HLR to aminoglycosides
Daptomycin plus another active agent[4,5]
Q/D plus another active agent[4,6]
Linezolid + another active agent

Q/D: Quinupristin/dalfopristin
[1] Doses up to 30 g/day may be considered
[2] Doses of 8-12 mg/kg are preferred
[3] If imipenem MIC < 32 µg/ml
[4] Agents with potential activity include ampicillin (even if resistant), tigecycline, doxycycline with rifampin, ampicillin, or fluoroquinolones (if susceptible to each agent)
[5] Agents that have been successfully used in case reports include tigecycline (three cases) and ampicillin (one case)
[6] The combination of Q/D plus doxycycline plus rifampin was successfully used in one patient

Treatment of less severe enterococcal infections

As previously mentioned, there are some cases in which enterococci are isolated from clinical samples, but may not need to be treated. This situation usually occurs in patients who have intravascular or urinary catheters, since these devices are usually the source of the infection. Although prospective studies have not specifically identified the subset of patients who have enterococci in the bloodstream or urine and may do well in the absence of specific antimicrobial therapy, clinical experience suggests that this group of patients may include: ***i***) immunocompetent patients without signs of infection (such as those with asymptomatic bacteriuria or bacteremia) in whom positive cultures harbor low bacterial burdens (for example, only one positive bottle in a set of many other blood cultures); ***ii***) cultures usually clear upon removal of the catheter; and ***iii***) patients do not have any risk for the development of endocarditis (that is, patients with prosthetic valves or those in whom prosthetic material was used for valve replacement, history of previous IE, congenital heart disease or patient with history of cardiac transplantation) (Baddour, et al., 2005).

In some individuals with true uncomplicated urinary tract infections (UTIs), the use of systemic agents may not be necessary, and antibiotics that concentrate in the urine may be useful in these settings. Fosfomycin and nitrofurantoin are two drugs that concentrate in the urine and still retain good *in vitro* activity against enterococci, and should be considered for the treatment of uncomplicated UTIs caused by enterococci. Fosfomycin has an FDA approval for the treatment of UTIs caused by vancomycin-susceptible enterococci, but not for VRE or any *E. faecium*. Furthermore, due to high concentrations of the amino-penicillins in the urine, ampicillin or amoxicilin may be useful in cases in which the ampicillin MICs of the enterococcal isolate is <128 µg/ml. It is important to emphasize that removal of the urinary catheter is of paramount importance for the successful eradication of the infective microorganisms in these cases (Arias & Murray, 2012).

Emergence of resistance during therapy

The malleability of enterococcal genomes, the intrinsic resistance of enterococci to several antimicrobial agents, and their abilities to recruit and disseminate antibiotic resistance determinants make these organisms a real challenge in clinical practice. One the main concerns that physicians face during the treatment of enterococcal disease is the possibility of developing resistance during therapy, which may eventually lead to therapeutic failures and, in several instances, contribute to the increased mortality of patients infected with these microorganisms. This issue is more relevant when the mechanism of resistance involves mutations of existing genes rather than the acquisition of new resistance determinants. Development of *in vivo* resistance to linezolid and daptomycin are two of the best examples of this phenomenon.

As previously mentioned, the level of linezolid resistance directly correlates with the number of rRNA genes mutated—the more mutated alleles, the higher the linezolid MIC. The link between mutations in the rRNA genes and the dynamics of emergence of linezolid resistance in *E. faecalis* during therapy was evaluated in a study using an artificial intestinal ecosystem in mono-associated gnotobiotic mice (Bourgeois-Nicolaos, Massias, Couson, Butel, Andremont, & Doucet-Populaire, 2007). This study provided compelling evidence that the selection of linezolid resistance in gnotobiotic mice was highly associated with concentrations of linezolid in the gut, which were directly correlated with the dose regimen used.

For example, in mice exposed to a regimen of 0.5 g/L of linezolid via oral gavage (which mimics the human dosing scheme), linezolid-resistant mutants were selected as early as two days after starting therapy and were identified up to 21 days after the initiation of treatment. However, mutations in only two out of the four rRNA alleles were identified in the *E. faecalis* derivatives with decreased linezolid susceptibility after 21 days of treatment with this regimen. Conversely, a lower-dose regimen of 0.05 g/L selected mutants more readily *in vivo* and mutants harbored mutations in all four copies of the 23S rRNA genes with higher MICs to linezolid than those identified in the higher-dose regimen (Bourgeois-Nicolaos, Massias, Couson, Butel, Andremont, & Doucet-Populaire, 2007).

The study supports the strong correlation between pharmacokinetic/pharmacodynamic parameters of linezolid and the emergence of *in vivo* resistance during therapy. Similarly, emergence of daptomycin resistance during therapy is an important cause of concern, as noted above. These data further emphasize the fact that the success of antimicrobial therapy against enterococci depends on many factors, including the optimization of the *in vitro* and *in vivo* activity of the antimicrobials, since enterococci have a remarkable ability to adapt to environmental stresses and respond to the "attack" of antibiotics.

Impact of antibiotic use on emergence of enterococcal nosocomial infections

Enterococci are commensals of the gastrointestinal (GI) tract of humans and animals, and, in the absence of antibiotics, establish a symbiotic relationship with the human microbiota. In normal circumstances, enterococci are found in low numbers in the colon, as compared with other members of the gut flora (such as anaerobes) (Murray, 2000). The "natural" balance in the microbiota becomes altered by the presence of antibiotics; the administration of cephalosporins and metronidazole, which are often used as part of an empiric regimen in critically ill patients, kills many Gram-negative and anaerobic bacteria in the intestine, but enterococci tend to survive, since these compounds have no potent activity against them. Moreover, some of the cephalosporins (such as ceftriaxone) are excreted in bile, a pharmacokinetic property that increases concentrations of this class of antibiotics in the gut, which maximizes the effect against the gut microbiota and promotes the expansion of enterococci (particularly VRE) in the GI tract (Donskey, et al., 2000).

The establishment of enterococci as the predominant flora in the gut of hospitalized patients has important clinical consequences, since it is from this biological niche that these microorganisms are capable of reaching the

bloodstream or persisting as colonizers that invade other tissues, such as the skin of hospitalized patients (Ubeda, et al., 2010). Indeed, once colonization is established, it may persist for prolonged periods. In a study that included 53 liver and kidney transplant recipients colonized by VRE, persistent colonization for more than three weeks was demonstrated in 66% of patients (Patiño, Chippaux, Courvalin, & Périchon, 2005). Moreover, VRE colonization with isolates that have similar pulsed field gel electrophoresis patterns to those of the initial colonizing strain has been shown to persist for more than a year (Baden, et al., 2001). Colonization of a patient in a medical unit is usually the main factor that triggers the dissemination of enterococcal strains (VRE) to other patients. Epidemiological factors that enhance this dissemination in hospital settings include prolonged hospitalization, physical proximity to patients infected or colonized by VRE, hospitalization in critical care units, and hospitalization in a room with a previous patient known to have had an infection or colonization with VRE, among others (Arias & Murray, 2012). The antibiotics that have been strongly associated with VRE colonization (*E. faecium*) in the GI tract include clindamycin, metronidazole, piperacillin-tazobactam, and second- or third-generation cephalosporins (Sullivan, Edlund, & Nord, 2001).

The actual molecular mediators that promote the colonization of VRE in the presence of antibiotics are unknown, but recent work using murine models of GI colonization has yielded important information to understand the dynamics of enterococcal colonization in the GI tract. Two recent pioneer studies (Brandl, et al., 2008; Kristich & Little, 2012) have been able to demonstrate that surface components of Gram-negative flora play an important role in maintaining the equilibrium between enterococci and their counterparts in the GI tract. Lipopolysaccharide (LPS) and flagellin of Gram-negative bacteria are capable of stimulating (via Toll-like receptors) the production of the lectin RegIIIγ (a C-type lectin) by intestinal epithelial cells. RegIIIγ is capable of killing a variety of Gram-positive organisms, including VRE. Therefore, upon the reduction in Gram-negative flora produced by the presence of antibiotics in the gut, the synthesis of REgIIIγ by the intestinal epithelium is drastically decreased. This effect directly promotes the overgrowth of VRE in the gut.

In mice, the predominance of VRE after the administration of metronidazole, neomycin, and vancomycin was observed for up to two months after discontinuation of the regimen (Ubeda, et al., 2010). Moreover, the high MICs of piperacillin and cephalosporins displayed by ampicillin-resistant *E. faecium* strongly contribute to maintaining VRE as the dominant flora of hospitalized patients in the presence of antibiotics. Ubeda et al. (Ubeda, et al., 2010) elegantly showed that critically ill patients (those undergoing bone marrow transplant due to hematological malignancy) in whom VRE had become the predominant intestinal flora after several courses of antimicrobials, were more likely to develop a bloodstream infection with these organisms, which supports the role of the GI tract as the source of infecting VRE strains in patients receiving antibiotics.

For the foreseeable future, MDR enterococci will continue to pose immense clinical challenges in hospitalized patients. The trends in the epidemiology of enterococcal infections suggest that MDR *E. faecium* may become the most common species isolated from hospitalized patients in the near future. As a result, novel therapeutic strategies focused on treating infections with these organisms are urgently needed.

References

Abadía Patiño L., Courvalin P., Perichon B. vanE gene cluster of vancomycin-resistant Enterococcus faecalis BM4405. Journal of Bacteriology. 2002;184(23):6457–6564. PubMed PMID: 12426332.

Alborn W. E. Jr, Allen N. E., Preston D. A. Daptomycin disrupts membrane potential in growing Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 1991;35(11):2282–2287. PubMed PMID: 1666494.

Andrews J., Ashby J., Jevons G., Marshall T., Lines N., Wise R. A comparison of antimicrobial resistance rates in Gram-positive pathogens isolated in the UK from October 1996 to January 1997 and October 1997 to January 1998. Journal of Antimicrobial Chemotherapy. 2000;45(3):285–293. PubMed PMID: 10702546.

Antony S. J., Ladner J., Stratton C. W., Raudales F., Dummer S. J. High-level aminoglycoside-resistant enterococcus causing endocarditis successfully treated with a combination of ampicillin, imipenem and vancomycin. Clinical Infectious Diseases. 1997;29(6):628–630. PubMed PMID: 9571747.

Arbeloa A., Segal H., Hugonnet J. E., Josseaume N., Dubost L., Brouard J. P., et al. Role of class A penicillin-binding proteins in PBP5-mediated beta-lactam resistance in Enterococcus faecalis. Journal of Bacteriology. 2004;186(5):1221–1228. PubMed PMID: 14973044.

Arias C. A., Murray B. E. The rise of the Enterococcus: beyond vancomycin resistance. Nature Reviews Microbiology. 2012;10(4):266–278. PubMed PMID: 22421879.

Arias C. A., Contreras G. A., Murray B. E. Emergence and management of drug-resistant enterococcal infections. Expert Review of Anti-infective Therapy. 2008;6:637–655. PubMed PMID: 18847403.

Arias C. A., Contreras G. A., Murray B. E. Management of multidrug-resistant enterococcal infections. Clinical Microbiology and Infection. 2010;16:555–562. PubMed PMID: 20569266.

Arias C. A., Mendes R. E., Stilwell M. G., Jones R. N., Murray B. E. Unmet needs and prospects for oritavancin in the management of vancomycin-resistant enterococcal infections. Clinical Infectious Diseases. 2012;54 Suppl 3:S233–S238. PubMed PMID: 22431854.

Arias C. A., Panesso D., McGrath D. M., Qin X., Mojica M. F., Miller C., et al. Genetic basis for in vivo daptomycin resistance in enterococci. The New England Journal of Medicine. 2011;365:892–900. PubMed PMID: 21899450.

Arias C. A., Singh K. V., Panesso D., Murray B. E. Evaluation of ceftobiprole medocaril against Enterococcus faecalis in a mouse peritonitis model. Journal of Antimicrobial Chemotherapy. 2007;60(3):594–598. PubMed PMID: 17606481.

Arias C. A., Singh K. V., Panesso D., Murray B. E. Time-kill and synergism studies of ceftobiprole against Enterococcus faecalis, including beta-lactamase-producing and vancomycin-resistant isolates. Antimicrobial Agents and Chemotherapy. 2007;51(6):2043–2047. PubMed PMID: 17438057.

Arias C. A., Torres H. A., Singh K. V., Panesso D., Moore J., Wanger A., et al. Failure of daptomycin monotherapy for endocarditis caused by an Enterococcus faecium strain with vancomycin-resistant and vancomycin-susceptible subpopulations and evidence of in vivo loss of the vanA gene cluster. Clinical Infectious Diseases. 2007;45(10):1343–1346. PubMed PMID: 17968832.

Arsène S., Leclercq R. Role of a qnr-Like Gene in the Intrinsic Resistance of Enterococcus faecalis to Fluoroquinolones. Antimicrobial Agents and Chemotherapy. 2007;51(9):3254–3258. PubMed PMID: 17620379.

Arthur M., Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrobial Agents and Chemotherapy. 1993;37(8):1563–1571. PubMed PMID: 8215264.

Arthur M., Quintiliani R. Jr. Regulation of VanA- and VanB-type glycopeptide resistance in enterococci. Antimicrobial Agents and Chemotherapy. 2001;45(2):375–381. PubMed PMID: 11158729.

Arthur M., Molinas C., Courvalin P. The VanS-VanR two-component regulatory system controls synthesis of depsipeptide peptidoglycan precursors in Enterococcus faecium 4147. Journal of Bacteriology. 1992;174(8):2582–2591. PubMed PMID: 1556077.

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. Journal of Bacteriology. 1993;175(1):117–127. PubMed PMID: 8380148.

Aslangul E., Massias L., Meulemans A., Chau F., Andremont A., Courvalin P., et al. Acquired gentamicin resistance by permeability impairment in Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2006;50(11):3615–3621. PubMed PMID: 17065620.

Baddour L. M., Wilson W. R., Bayer A. S., Fowler V. G. Jr, Bolger A. F., Levison M. E., et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. Circulation. 2005;111:e394–e434. PubMed PMID: 15956145.

Baden L. R., Thiemke W., Skolnik A., Chambers R., Strymish J., Moellering R. C. Jr, et al. Prolonged colonization with vancomycin-resistant Enterococcus faecium in long-term care patients and the significance of "clearance". Clinical Infectious Diseases. 2001;33(10):1654–1660. PubMed PMID: 11595985.

Baptista M., Rodrigues P., Depardieu F., Courvalin P., Arthur M. Single-cell analysis of glycopeptide resistance gene expression in teicoplanin-resistant mutants of a VanB-type Enterococcus faecalis. Molecular Microbiology. 1999;32(1):17–28. PubMed PMID: 10216856.

Berdal J. E., Eskesen A. Short-term success, but long-term treatment failure with linezolid for enterococcal endocarditis. Clinical Infectious Diseases. 2008;40(9):765–766. PubMed PMID: 18609208.

Bethea J. A., Walko C. M., Targos P. A. Treatment of vancomycin-resistant enterococcus with quinupristin/dalfopristin and high-dose ampicillin. Annals of Pharmacotherapy. 2004;38(6):989–991. PubMed PMID: 15100393.

Birmingham M. C., Rayner C. R., Meagher A. K., Flavin S. M., Batts D. H., Schentag J. J. Linezolid for the treatment of multidrug-resistant, gram-positive infections: experience from a compassionate-use program. Clinical Infectious Diseases. 2003;36(2):159–168. PubMed PMID: 12522747.

Bjørkeng E., Rasmussen G., Sundsfjord A., Sjöberg L., Hegstad K., Söderquist B. Clustering of polyclonal VanB-type vancomycin-resistant Enterococcus faecium in a low-endemic area was associated with CC17-genogroup strains harbouring transferable vanB2-Tn5382 and pRUM-like repA containing plasmids with axe-txe plasmid addiction system. APMIS. 2011;119(4-5):247–258. PubMed PMID: 21492224.

Bourdon N., Fines-Guyon M., Thiolet J. M., Maugat S., Coignard B., Leclercq R., et al. Changing trends in vancomycin-resistant enterococci in French hospitals, 2001-08. Journal of Antimicrobial Chemotherapy. 2011;66(4):713–721. PubMed PMID: 21393182.

Bourgeois-Nicolaos N., Massias L., Couson B., Butel M. J., Andremont A., Doucet-Populaire F. Dose dependence of emergence of resistance to linezolid in Enterococcus faecalis in vivo. The Journal of Infectious Diseases. 2007;195(10):1480–1488. PubMed PMID: 17436228.

Bourgogne A., Garsin D. A., Qin X., Singh K. V., Sillanpaa J., Yerrapragada S., et al. Large scale variation in Enterococcus faecalis illustrated by the genome analysis of strain OG1RF. Genome Biology. 2008;9(7):R110. PubMed PMID: 18611278.

Boyd D. A., Willey B. M., Fawcett D., Gillani N., Mulvey M. R. Molecular characterization of Enterococcus faecalis N06-0364 with low-level vancomycin resistance harboring a novel D-Ala-D-Ser gene cluster, vanL. Antimicrobial Agents and Chemotherapy. 2008;52(7):2667–2672. PubMed PMID: 18458129.

Brandl K., Plitas G., Mihu C. N., Ubeda C., Jia T., Fleisher M., et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature. 2008;455(7214):804–807. PubMed PMID: 18724361.

Brandt C. M., Rouse M. S., Laue N. W., Stratton C. W., Wilson W. R., Steckelberg J. M. Effective treatment of multidrug-resistant enterococcal experimental endocarditis with combinations of cell wall-active agents. The Journal of Infectious Diseases. 1996;173(4):909–913. PubMed PMID: 8603970.

Bruand C., Chatelier L., Ehrlich S. D., Janniere L. A fourth class of theta-replicating plasmids: the pAMß1 family from Gram-positive bacteria. Proceedings of the National Academy of Science. 1993;90:11688–11672.

Canepari P., Lleò M. M., Cornaglia G., Fontana R., Satta G. In Streptococcus faecium penicillin-binding protein 5 alone is sufficient for growth at sub-maximal but not at maximal rate. Microbiology. 1986;132(3):625–631. PubMed PMID: 3734749.

Cantón R., Ruiz-Garbajosa P., Chaves R. L., Johnson A. P. A potential role for daptomycin in enterococcal infections: what is the evidence? Journal of Antimicrobial Chemotherapy. 2010;65(6):1126–1136. PubMed PMID: 20363805.

Carias L. L., Rudin S. D., Donskey C. J., Rice L. B. Genetic linkage and cotransfer of a novel, vanB-containing transposon (Tn5382) and a low-affinity penicillin-binding protein 5 gene in a clinical vancomycin-resistant Enterococcus faecium isolate. Journal of Bacteriology. 1998;180(17):4426–4434. PubMed PMID: 9721279.

Carmeli Y., Eliopoulos G. M., Samore M. H. Antecedent treatment with different antibiotic agents as a risk factor for vancomycin-resistant Enterococcus. Emerging Infectious Diseases. 2002;8(8):802–807. PubMed PMID: 12141965.

Celli J., Trieu-Cuot P. Circularization of Tn916 is required for expression of the transposon-encoded transfer functions: characterization of long tetracycline-inducible transcripts reading through the attachment site. Molecular Microbiology. 1998;28(1):103–117. PubMed PMID: 9593300.

Cervera C., Castañeda X., Pericas J. M., Del Río A., de la Maria C. G., Mestres C., et al. Clinical utility of daptomycin in infective endocarditis caused by Gram-positive cocci. International Journal of Antimicrobial Agents. 2011;38(5):365–370. PubMed PMID: 21420835.

Chirugi V. A., Oster S. E., Goldber A. A., McCabe R. E. Nosocomial acquisition of ß-lactamase-negative, ampicillin-resistant enterococcus. Archives of Internal Medicine. 1992;152(7):1457–1461. PubMed PMID: 1627025.

Chow J. W. Aminoglycoside resistance in enterococci. Clinical Infectious Diseases. 2000;31(2):586–589. PubMed PMID: 10987725.

Christie C., Hammond J., Reising S., Evans-Patterson J. Clinical and molecular epidemiology of enterococcal bacteremia in a pediatric teaching hospital. Journal of Pediatrics. 1994;125(3):392–399. PubMed PMID: 8071746.

Christie P. J., Korman R. Z., Zahler S. A., Adsit J. C., Dunny G. M. Two conjugation systems associated with plasmid pCF10: identification of a conjugative transposon that transfers between Streptococcus faecalis and Bacillus subtilis. Journal of Bacteriology. 1987;169(6):2529–2936. PubMed PMID: 3034859.

Clancy J., Petitpas J., Dib-Hajj F., Yuan W., Cronan M., Kamath A. V., et al. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from Streptococcus pyogenes. Molecular Microbiology. 1996;22(5):867–879. PubMed PMID: 8971709.

Clewell D. B. Properties of Enterococcus faecalis plasmid pAD1, a member of a widely disseminated family of pheromone-responding, conjugative, virulence elements encoding cytolysin. Plasmid. 2007;58(3):205–227. PubMed PMID: 17590438.

Clewell D. B., Flannagan S. E., Jaworski D. D. Unconstrained bacterial promiscuity: the Tn916-Tn1545 family of conjugative transposons. Trends in Microbiology. 1995;3(6):229–236. PubMed PMID: 7648031.

Clewell, D. B., Flannagan, S. E., Zitzow, L. O., Su, Y. A., He, P., Senghas, E., et al. (1991). Properties of conjugative transposon Tn916. In G. M. Dunny, P. Patrick, & L. L. Cleary (Eds.), *Genetics and molecular biology of streptococci, lactococci, and enterococci* (pp. 39-44). Washington, D. C.: American Society for Microbiology.

Clinical and Laboratory Standards Institute. (2012). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement.* Wayne: Clinical and Laboratory Standards Institute.

Cohen S. H., Gerding D. N., Johnson S., Kelly C. P., Loo V. G., McDonald L. C., et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infection Control and Hospital Epidemiology. 2010;31(5):431–455. PubMed PMID: 20307191.

Comenge Y., Quintiliani R. Jr, Li L., Dubost L., Brouard J.-P., Hugonnet J.-E., et al. The CroRS two-component regulatory system is required for intrinsic beta-lactam resistance in Enterococcus faecalis. Journal of Bacteriology. 2003;185(24):7184–7192. PubMed PMID: 14645279.

Coque T. M., Tomayko J. F., Ricke S. C., Okhyusen P. C., Murray B. E. Vancomycin-resistant enterococci from nosocomial, community, and animal sources. Antimicrobial Agents and Chemotherapy. 1996;40(11):2605–2609. PubMed PMID: 8913473.

Costa Y., Galimand M., Leclercq R., Duval J., Courvalin P. Characterization of the chromosomal aac(6′)-Ii gene specific for Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 1993;37(9):1896–1903. PubMed PMID: 8239603.

Courvalin P. Genetics of glycopeptide resistance in gram-positive pathogens. International Journal of Medical Microbiology. 2005;294(8):479–486. PubMed PMID: 15790292.

Courvalin P. Vancomycin resistance in gram-positive cocci. Clinical Infectious Diseases. 2006;42 Suppl 1:S25–S34. PubMed PMID: 16323116.

Courvalin P., Carlier C. Tn1545: a conjugative shuttle transposon. Molecular and General Genetics. 1987;206(2):259–264. PubMed PMID: 3035335.

Crank C. W., Scheetz M. H., Brielmaier B., Rose W. E., Patel G. P., Ritchie D. J., et al. Comparison of outcomes from daptomycin or linezolid treatment for vancomycin-resistant enterococcal bloodstream infection: A retrospective, multicenter, cohort study. Clinical Therapeutics. 2010;32(10):1713–1719. PubMed PMID: 21194593.

Cremniter J., Mainardi J. L., Josseaume N., Quincampoix J. C., Dubost L., Hugonnet J. E., et al. Novel mechanism of resistance to glycopeptide antibiotics in Enterococcus faecium. The Journal of Biological Chemistry. 2006;281(43):32254–32262. PubMed PMID: 16943188.

Currie B. P., Lemos-Filho L. Evidence for biliary excretion of vancomycin into stool during intravenous therapy: potential implications for rectal colonization with vancomycin-resistant enterococci. Antimicrobial Agents and Chemotherapy. 2004;48(11):4427–4429. PubMed PMID: 15504873.

Dahl K. H., Lundblad E. W., Rokenes T. P., Olsvik O., Sundsfjord A. Genetic linkage of the vanB2 gene cluster to Tn5382 in vancomycin-resistant enterococci and characterization of two novel insertion sequences. Microbiology. 2000;146(Pt 6):1469–1479. PubMed PMID: 10846225.

Dahl K. H., Mater D. D., Flores M. J., Johnsen P. J., Midtvedt T., Corthier G., et al. Transfer of plasmid and chromosomal glycopeptide resistance determinants occurs more readily in the digestive tract of mice than in vitro and exconjugants can persist stably in vivo in the absence of glycopeptide selection. Journal of Antimicrobial Chemotherapy. 2007;59(3):478–486. PubMed PMID: 17283034.

Davis D. R., McAlpine J. B., Pazoles C. J., Talbot M. K., Alder E. A., White C., et al. Enterococcus faecalis multi-drug resistance transporters: application for antibiotic discovery. Journal of Molecular Microbiology and Biotechnology. 2001;3(2):179–184. PubMed PMID: 11321571.

de Regt M. J., van der Wagen L. E., Top J., Blok H. E., Hopmans T. E., Dekker A. W., et al. High acquisition and environmental contamination rates of CC17 ampicillin-resistant Enterococcus faecium in a Dutch hospital. Journal of Antimicrobial Chemotherapy. 2008;62(6):1401–1406. PubMed PMID: 18812427.

Depardieu F., Courvalin P., Kolb A. Binding sites of VanRB and sigma70 RNA polymerase in the VanB vancomycin resistance operon of Enterococcus faecium BM4524. Molecular Microbiology. 2005;57(2):550–564. PubMed PMID: 15978084.

Depardieu F., Courvalin P., Msadek T. A six amino acid deletion, partially overlapping the VanSB G2 ATP-binding motif, leads to constitutive glycopeptide resistance in VanB-type Enterococcus faecium. Molecular Microbiology. 2003;50(3):1069–1083. PubMed PMID: 14617162.

Depardieu F., Kolbert M., Pruul H., Bell J., Courvalin P. VanD-type vancomycin-resistant Enterococcus faecium and Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2004;48(10):3892–3904. PubMed PMID: 15388450.

Depardieu F., Podglajen I., Leclercq R., Collatz E., Courvalin P. Modes and modulations of antibiotic resistance gene expression. Clinical Microbiology Reviews. 2007;20(1):79–114. PubMed PMID: 17223624.

Descheemaeker P. R., Chapelle S., Devriese L. A., Butaye P., Vandamme P., Goossens H. Comparison of glycopeptide-resistant Enterococcus faecium isolates and glycopeptide resistance genes of human and animal origin. Antimicrobial Agents and Chemotherapy. 1999;43(8):2032–2037. PubMed PMID: 10428931.

Diaz L., Kiratisin P., Mendes R. E., Panesso D., Singh K. V., Arias C. A. Transferable Plasmid-Mediated Resistance to Linezolid Due to cfr in a Human Clinical Isolate of Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2012;56(7):3917–3922. PubMed PMID: 22491691.

Dina J., Malbruny B., Leclercq R. Nonsense mutations in the lsa-like gene in Enterococcus faecalis isolates susceptible to lincosamides and streptogramins A. Antimicrobial Agents and Chemotherapy. 2003;47(7):2307–2309. PubMed PMID: 12821484.

Donskey C. J., Chowdhry T. K., Hecker M. T., Hoyen C. K., Hanrahan J. A., Hujer A. M., et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. The New England Journal of Medicine. 2000;343(26):1925–1932. PubMed PMID: 11136263.

Donskey C. J., Hanrahan J. A., Hutton R. A., Rice L. B. Effect of parenteral antibiotic administration on establishment of colonization with vancomycin-resistant Enterococcus faecium in the mouse gastrointestinal tract. Journal of Infectious Diseases. 1999;180(2):384–390. PubMed PMID: 10395853.

Ehrenfeld E. E., Clewell D. B. Transfer functions of the Streptococcus faecalis plasmid pAD1: organization of plasmid DNA encoding response to sex pheromone. Journal of Bacteriology. 1987;169(8):3473–3481. PubMed PMID: 3038841.

El-Khoury J., Fishman J. A. Linezolid in the treatment of vancomycin-resistant Enterococcus faecium in solid organ transplant recipients: report of a multicenter compassionate-use trial. Transplant Infectious Disease. 2003;5(3):121–125. PubMed PMID: 14617299.

Enne V. I., Delsol A. A., Roe J. M., Bennett P. M. Rifampicin resistance and its fitness cost in Enterococcus faecium. Journal of Antimicrobial Chemotherapy. 2004;53(2):203–207. PubMed PMID: 14688044.

Erlandson K. M., Sun J., Iwen P. C., Rupp M. E. Impact of the more-potent antibiotics quinupristin-dalfopristin and linezolid on outcome measure of patients with vancomycin-resistant Enterococcus bacteremia. Clinical Infectious Diseases. 2008;46(1):30–36. PubMed PMID: 18171210.

Falagas M. E., Manta K. G., Ntziora F., Vardakas K. Z. Linezolid for the treatment of patients with endocarditis: a systematic review of the published evidence. Journal of Antimicrobial Chemotherapy. 2006;58(2):273–280. PubMed PMID: 16735427.

Fines M., Perichon B., Reynolds P., Sahm D. F., Courvalin P. VanE, a new type of acquired glycopeptide resistance in Enterococcus faecalis BM4405. Antimicrobial Agents and Chemotherapy. 1999;43(9):2161–2164. PubMed PMID: 10471558.

Foucault M. L., Depardieu F., Courvalin P., Grillot-Courvalin C. Inducible expression eliminates the fitness cost of vancomycin resistance in enterococci. Proceedings of the National Academy of Sciences. 2010;107(39):16964–16969. PubMed PMID: 20833818.

Franke A. E., Clewell D. B. Evidence for a chromosome-borne resistance transposon (Tn916) in Streptococcus faecalis that is capable of "conjugal" transfer in the absence of a conjugative plasmid. Journal of Bacteriology. 1981;145(1):494–502. PubMed PMID: 6257641.

Friedman L., Alder J. D., Silverman J. A. Genetic changes that correlate with reduced susceptibility to daptomycin in Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2006;50(6):2137–2145. PubMed PMID: 16723576.

Galimand M., Schmitt E., Panvert M., Desmolaize B., Douthwaite S., Mechulam Y., et al. Intrinsic resistance to aminoglycosides in Enterococcus faecium is conferred by the 16S rRNA m5C1404-specific methyltransferase EfmM. RNA. 2011;17(2):251–262. PubMed PMID: 21159796.

Galloway-Peña J. R., Rice L. B., Murray B. E. Analysis of PBP5 of early U.S. isolates of Enterococcus faecium: sequence variation alone does not explain increasing ampicillin resistance over time. Antimicrobial Agents and Chemotherapy. 2011;55(7):3272–3277. PubMed PMID: 21576454.

Galloway-Peña J., Roh J. H., Latorre M., Qin X., Murray B. E. Genomic and SNP analyses demonstrate a distant separation of the hospital and community-associated clades of Enterococcus faecium. PLoS One. 2012;7(1):e30187. PubMed PMID: 22291916.

Garnier F., Taourit S., Glaser P., Courvalin P., Galimand M. Characterization of transposon Tn1549, conferring VanB-type resistance in Enterococcus spp. Microbiology. 2000;146:1481–1489. PubMed PMID: 10846226.

Gavaldà J., Len O., Miró J. M., Muñoz P., Montejo M., Alarcón A., et al. Brief communication: treatment of Enterococcus faecalis endocarditis with ampicillin plus ceftriaxone. Annals of Internal Medicine. 2007;146(8):574–579. PubMed PMID: 17438316.

Goffin C., Ghuysen J. M. Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. Microbiology and Molecular Biology Reviews. 1998;62(4):1079–1093. PubMed PMID: 9841666.

Gonzales R. D., Schrekenberger P. C., Graham M. B., Kelkar S., DenBesten K., Quinn J. P. Infections due to vancomycin-resistant Enterococcus faecium resistant to linezolid. Lancet. 2001;357(9263):1179. PubMed PMID: 11323048.

Grayson M. L., Eliopoulos G. M., Wennersten C. B., Ruoff K. L., De Girolami P. C., Ferraro M. J., et al. Increasing resistance to ß-lactam antibiotics among clinical isolates of Enterococcus faecium: a 22-year review at one institution. Antimicrobial Agents and Chemotherapy. 1991;35(11):2180–2184. PubMed PMID: 1803989.

Gryczan T. J., Grandi G., Hahn J., Grandi R., Dubnau D. Conformational alteration of mRNA structure and the posttranscriptional regulation of erythromycin-induced drug resistance. Nucleic Acids Research. 1980;8(24):6081–6097. PubMed PMID: 6162157.

Hall A. D., Steed M. E., Arias C. A., Murray B. E., Rybak M. J. Evaluation of Standard- and High-Dose Daptomycin versus Linezolid against Vancomycin-Resistant Enterococcus Isolates in an In Vitro Pharmacokinetic/Pharmacodynamic Model with Simulated Endocardial Vegetations. Antimicrobial Agents and Chemotherapy. 2012;56(6):3174–3180. PubMed PMID: 22470111.

Hancock L. E., Perego M. Systematic inactivation and phenotypic characterization of two-component signal transduction systems of Enterococcus faecalis V583. Journal of Bacteriology. 2004;186(23):7951–7958. PubMed PMID: 15547267.

Hancock L., Perego M. Two-component signal transduction in Enterococcus faecalis. Journal of Bacteriology. 2002;184(21):5819–5825. PubMed PMID: 12374813.

Hartmann C., Peter C., Hermann E., Ure B., Sedlacek L., Hansen G., et al. Successful treatment of vancomycin-resistant Enterococcus faecium ventriculitis with combined intravenous and intraventricular chloramphenicol in a newborn. Journal of Medical Microbiology. 2010;59(Pt 11):1371–1374. PubMed PMID: 20634329.

Heaton M. P., Handwerger S. Conjugative mobilization of a vancomycin resistance plasmid by a putative Enterococcus faecium sex pheromone response plasmid. Microbial Drug Resistance. 1995;1(2):177–183. PubMed PMID: 9158753.

Heaton M. P., Discotto L. F., Pucci M. J., Handwerger S. Mobilization of vancomycin resistance by transposon-mediated fusion of a VanA plasmid with an Enterococcus faecium sex pheromone-response plasmid. Gene. 1996;171(1):9–17. PubMed PMID: 8675038.

Hegde S. S., Vetting M. W., Mitchenall L. A., Maxwell A., Blanchard J. S. Structural and biochemical analysis of the pentapeptide repeat protein EfsQnr, a potent DNA gyrase inhibitor. Antimicrobial Agents and Chemotherapy. 2011;55(1):110–117. PubMed PMID: 20937785.

Hellinger W. C., Rouse M. S., Rabadan P. M., Henry N. K., Steckelberg J. M., Wilson W. R. Continuous intravenous versus intermittent ampicillin therapy of experimental endocarditis caused by aminoglycoside-resistant enterococci. Antimicrobial Agents and Chemotherapy. 1992;36(6):1272–1275. PubMed PMID: 1416826.

Hidron A. I., Edwards J. R., Patel J., Horan T. C., Sievert D. M., Pollock D. A., et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infection Control and Hospital Epidemiology. 2008;29(11):996–1011. PubMed PMID: 18947320.

Hodel-Christian S. L., Murray B. E. Characterization of the gentamicin resistance transposon Tn5281 from Enterococcus faecalis and comparison to staphylococcal transposons Tn4001 and Tn4031. Antimicrobial Agents and Chemotherapy. 1991;35(6):1147–1152. PubMed PMID: 1656854.

Hong H.-J., Hutchings M. I., Buttner M. J. Vancomycin resistance VanS/VanR two-component systems. Advances in Experimental Medicine and Biology. 2008;631:200–213. PubMed PMID: 18792691.

Jaspan H. B., Brothers A. W., Campbell A. J., McGuire J. K., Browd S. R., Manley T. J., et al. Multidrug-resistant Enterococcus faecium meningitis in a toddler: characterization of the organism and successful treatment with intraventricular daptomycin and intravenous tigecycline. The Pediatric Infectious Disease Journal. 2010;29(4):379–381. PubMed PMID: 20010311.

Jawetz E., Sonne M. Penicillin-streptomycin treatment of enterococcal endocarditis: a reevaluation. New England Journal of Medicine. 1966;274:710–715. PubMed PMID: 5908873.

Jensen L. B., Frimodt-Møller N., Aarestrup F. M. Presence of erm gene classes in Gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiology Letters. 1999;170(1):151–158. PubMed PMID: 9919664.

Jonas B. M., Murray B. E., Weinstock G. M. Characterization of EmeA, a NorA homolog and multidrug resistance efflux pump, in Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2001;45(12):3574–3579. PubMed PMID: 11709342.

Jung Y. H., Shin E. S., Kim O., Yoo J. S., Lee K. M., Yoo J. I., et al. Characterization of two newly identified genes, vgaD and vatH, [corrected] conferring resistance to streptogramin A in Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2010;54(11):4744–4749. PubMed PMID: 20713681.

Kak, V., & Chow, J. W. (2002). Acquired antibiotic resistances in enterococci. In M. S. Gilmore, D. B. Clewell, P. Courvalin, G. M. Dunny, B. E. Murray, & L. B. Rice (Eds.), *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance* (pp. 355-383). Washington, D. C.: American Society for Microbiology.

Kak V., Donabedian S. M., Zervos M. J., Kariyama R., Kumon H., Chow J. W. Efficacy of ampicillin plus arbekacin in experimental rabbit endocarditis caused by an Enterococcus faecalis strain with high-level gentamicin resistance. Antimicrobial Agents and Chemotherapy. 2000;44(9):2545–2546. PubMed PMID: 10952613.

Kak V., You I., Zervos M. J., Kariyama R., Kumon H., Chow J. W. In-vitro activity of the combination of ampicillin and arbekacin against high-level gentamicin-resistant enterococci. Journal of Antimicrobial Chemotherapy. 2000;37(4):279–299.

Kinnebrew M. A., Ubeda C., Zenewicz L. A., Smith N., Flavell R. A., Pamer E. G. Bacterial flagellin stimulates Toll-like receptor 5-dependent defense against vancomycin-resistant Enterococcus infection. The Journal of Infectious Diseases. 2010;201(4):534–543. PubMed PMID: 20064069.

Kristich C. J., Little J. L. Mutations in the beta Subunit of RNA Polymerase Alter Intrinsic Cephalosporin Resistance in Enterococci. Antimicrobial Agents and Chemotherapy. 2012;56(4):2022–2027. PubMed PMID: 22290974.

Kristich C. J., Little J. L., Hall C. L., Hoff J. S. Reciprocal regulation of cephalosporin resistance in Enterococcus faecalis. MBio. 2011;2(6):e00199–e00111. PubMed PMID: 22045988.

Kristich C. J., Wells C. L., Dunny G. M. A eukaryotic-type Ser/Thr kinase in Enterococcus faecalis mediates antimicrobial resistance and intestinal persistence. Proceedings of the National Academy of Sciences. 2007;104(9):3508–3513. PubMed PMID: 17360674.

Kuch A., Willems R. J., Werner G., Coque T. M., Hammerum A. M., Sundsfjord A., et al. Insight into antimicrobial susceptibility and population structure of contemporary human Enterococcus faecalis isolates from Europe. Journal of Antimicrobial Chemotherapy. 2012;67(3):551–558. PubMed PMID: 22207599.

Lautenbach E., Schuster M. B., Bilker W. B., Brennan P. J. The role of chloramphenicol in the treatment of bloodstream infection due to vancomycin-resistant Enterococcus. Clinical Infectious Diseases. 1998;27(5):1259–1565. PubMed PMID: 9827280.

Laverde Gomez J. A., van Schaik W., Freitas A. R., Coque T. M., Weaver K. E., Francia M. V., et al. A multiresistance megaplasmid pLG1 bearing a hylEfm genomic island in hospital Enterococcus faecium isolates. International Journal of Medical Microbiology. 2011;301(2):165–175. PubMed PMID: 20951641.

Le Breton Y., Muller C., Auffray Y., Rincé A. New insights into the Enterococcus faecalis CroRS two-component system obtained using a differential-display random arbitrarily primed PCR approach. Applied and Environmental Microbiology. 2007;73(11):3738–3741. PubMed PMID: 17434998.

Lebreton F., Depardieu F., Bourdon N., Fines-Guyon M., Berger P., Camiade S., et al. D-Ala-d-Ser VanN-type transferable vancomycin resistance in Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2011;55(10):4606–4612. PubMed PMID: 21807981.

Lee E.-W., Huda M. N., Kuroda T., Mizushima T., Tsuchiya T. EfrAB, an ABC multidrug efflux pump in Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2003;47(12):3733–3738. PubMed PMID: 14638474.

Lefort A., Saleh-Mghir A., Garry L., Carbon C., Fantin B. Activity of LY333328 combined with gentamicin in vitro and in rabbit experimental endocarditis due to vancomycin-susceptible or -resistant Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2000;44:3017–3021. PubMed PMID: 11036016.

Linden P. K., Moellering R. C. Jr, Wood C. A., Rehm S. J., Flaherty J., Bompart F., et al. Treatment of vancomycin-resistant Enterococcus faecium infections with quinupristin/dalfopristin. Clinical Infectious Diseases. 2001;33(11):1816–1823. PubMed PMID: 11668430.

Lobritz M., Hutton-Thomas R., Marshall S., Rice L. B. Recombination proficiency influences frequency and locus of mutational resistance to linezolid in Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2003;47(10):3318–3320. PubMed PMID: 14506047.

Lu J. J., Chang T. Y., Perng C. L., Lee S. Y. The vanB2 gene cluster of the majority of vancomycin-resistant Enterococcus faecium isolates from Taiwan is associated with the pbp5 gene and is carried by Tn5382 containing a novel insertion sequence. Antimicrobial Agents and Chemotherapy. 2005;49(9):3739–3939. PubMed PMID: 16127076.

Lyon B. R., May J. W., Skurray R. A. Tn4001: a gentamicin and kanamycin resistance transposon in Staphylococcus aureus. Molecular and General Genetics. 1984;193(3):554–556. PubMed PMID: 6323927.

Magnet S., Arbeloa A., Mainardi J. L., Hugonnet J. E., Fourgeaud M., Dubost L., et al. Specificity of L,D-transpeptidases from Gram-positive bacteria producing different peptidoglycan chemotypes. The Journal of Biological Chemistry. 2007;282(18):13151–13159. PubMed PMID: 17311917.

Mainardi J. L., Fourgeaud M., Hugonnet J. E., Dubost L., Brouard J. P., Ouazzani J., et al. A novel peptidoglycan cross-linking enzyme for a beta-lactam-resistant transpeptidation pathway. The Journal of Biological Chemistry. 2005;280(46):38146–38152. PubMed PMID: 16144833.

Mainardi J. L., Gutmann L., Acar J. F., Goldstein F. W. Synergistic effect of amoxicillin and cefotaxime against Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 1995;39(9):1984–1987. PubMed PMID: 8540703.

Mainardi J. L., Hugonnet J. E., Rusconi F., Fourgeaud M., Dubost L., Moumi A. N., et al. Unexpected inhibition of peptidoglycan LD-transpeptidase from Enterococcus faecium by the beta-lactam imipenem. The Journal of Biological Chemistry. 2007;282(42):30414–30422. PubMed PMID: 17646161.

Mainardi J. L., Legrand R., Arthur M., Schoot B., van Heijenoort J., Gutmann L. Novel mechanism of beta-lactam resistance due to bypass of DD-transpeptidation in Enterococcus faecium. The Journal of Biological Chemistry. 2000;275(22):16490–16496. PubMed PMID: 10748168.

Mainardi J. L., Morel V., Fourgeaud M., Cremniter J., Blanotq D., Legrand R., et al. Balance between two transpeptidation mechanisms determines the expression of beta-lactam resistance in Enterococcus faecium. The Journal of Biological Chemistry. 2002;277(39):35801–35807. PubMed PMID: 12077139.

Manson J. M., Hancock L. E., Gilmore M. S. Mechanism of chromosomal transfer of Enterococcus faecalis pathogenicity island, capsule, antimicrobial resistance, and other traits. Proceedings of the National Academy of Sciences. 2010;107(27):12269–12274. PubMed PMID: 20566881.

Marshall S. H., Donskey C. J., Hutton-Thomas R., Salata R. A., Rice L. B. Gene dosage and linezolid resistance in Enterococcus faecium and Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2002;46(10):3334–3336. PubMed PMID: 12234875.

Martínez-Martínez L., Pascual A., Jacoby G. A. Quinolone resistance from a transferable plasmid. Lancet. 1998;351(9105):797–799. PubMed PMID: 9519952.

Mascher T., Helmann J. D., Unden G. Stimulus perception in bacterial signal-transducing histidine kinases. Microbiology and Molecular Biology Reviews. 2006;70(4):910–938. PubMed PMID: 17158704.

Mascio C. T., Alder J. D., Silverman J. A. Bactericidal action of daptomycin against stationary-phase and nondividing Staphylococcus aureus cells. Antimicrobial Agents and Chemotherapy. 2007;51(12):4255–4260. PubMed PMID: 17923487.

Matsumura S., Simor A. E. Treatment of endocarditis due to vancomycin-resistant Enterococcus faecium with quinupristin/dalfopristin, doxycycline, and rifampin: a synergistic drug combination. Clinical Infectious Diseases. 1998;27(6):1554–1556. PubMed PMID: 9868693.

Mave V., Garcia-Diaz J., Islam T., Hasbun R. Vancomycin-resistant enterococcal bacteraemia: is daptomycin as effective as linezolid? Journal of Antimicrobial Chemotherapy. 2009;64:175–180. PubMed PMID: 19423543.

Mendes R. E., Woolsey L. N., Farrell D. J., Sader H. S., Jones R. N. Oritavancin activity against vancomycin-susceptible and vancomycin-resistant Enterococci with molecularly characterized glycopeptide resistance genes recovered from bacteremic patients, 2009-2010. Antimicrobial Agents and Chemotherapy. 2011;56:1639–1642. PubMed PMID: 22183169.

Mir M., Asong J., Li X., Cardot J., Boons G. J., Husson R. N. The extracytoplasmic domain of the Mycobacterium tuberculosis Ser/Thr kinase PknB binds specific muropeptides and is required for PknB localization. PLOS Pathogens. 2011;7(7):e1002182. PubMed PMID: 21829358.

Moellering R. C. Jr, Weinberg A. N. Studies on antibiotic syngerism against enterococci. II. Effect of various antibiotics on the uptake of 14 C-labeled streptomycin by enterococci. The Journal of Clinical Investigation. 1971;50(12):2580–2584. PubMed PMID: 5001959.

Moellering R. C. Jr, Linden P. K., Reinhardt J., Blumberg E. A., Bompart F., Talbot G. H. The efficacy and safety of quinupristin/dalfopristin for the treatment of infections caused by vancomycin-resistant Enterococcus faecium. Synercid Emergency-Use Study Group. Journal of Antimicrobial Chemotherapy. 1999;44(2):251–261. PubMed PMID: 10473233.

Mohr J. F., Friedrich L. V., Yankelev S., Lamp K. C. Daptomycin for the treatment of enterococcal bacteraemia: results from the Cubicin Outcomes Registry and Experience (CORE). International Journal of Antimicrobial Agents. 2009;33(6):543–548. PubMed PMID: 19201165.

Montero C. I., Stock F., Murray P. R. Mechanisms of resistance to daptomycin in Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2008;52(3):1167–1170. PubMed PMID: 18180351.

Muller C., Le Breton Y., Morin T., Benachour A., Auffray Y., Rincé A. The response regulator CroR modulates expression of the secreted stress-induced SalB protein in Enterococcus faecalis. Journal of Bacteriology. 2006;188(7):2636–2645. PubMed PMID: 16547051.

Murray B. E. The life and times of the Enterococcus. Clinical Microbiology Reviews. 1990;3(1):46–65. PubMed PMID: 2404568.

Murray B. E. Vancomycin-resistant enterococcal infections. The New England Journal of Medicine. 2000;342:710–721. PubMed PMID: 10706902.

Murray B. E., Mederski-Samoraj B., Foster S. K., Brunton J. L., Harford P. In vitro studies of plasmid-mediated penicillinase from Streptococcus faecalis suggest a staphylococcal origin. The Journal of Clinical Investigation. 1986;77(1):289–293. PubMed PMID: 3080475.

Norris A. H., Reilly J. P., Edelstein P. H., Brennan P. J., Schuster M. G. Chloramphenicol for the treatment of vancomycin-resistant enterococcal infections. Clinical Infectious Diseases. 1995;20(5):1137–1144. PubMed PMID: 7619989.

Okamoto R., Okubo T., Inoue M. Detection of genes regulating ß-lactamase production in Enterococcus faecalis and Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 1996;40(11):2550–2554. PubMed PMID: 8913462.

Ono S., Muratani T., Mastumoto T. Mechanisms of resistance to imipenem and ampicillin in Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2005;49(7):2954–2958. PubMed PMID: 15980374.

Oyamada Y., Ito H., Fujimoto K., Asada R., Niga T., Okamoto R., et al. Combination of known and unknown mechanisms confers high-level resistance to fluoroquinolones in Enterococcus faecium. Journal of Medical Microbiology. 2006;55(Pt 6):729–736. PubMed PMID: 16687591.

Oyamada Y., Ito H., Inoue M., Yamagishi J.-i. Topoisomerase mutations and efflux are associated with fluoroquinolone resistance in Enterococcus faecalis. Journal of Medical Microbiology. 2006;55(10):1395–1401. PubMed PMID: 17005789.

Palmer K. L., Daniel A., Hardy C., Silverman J., Gilmore M. S. Genetic basis for daptomycin resistance in enterococci. Antimicrobial Agents and Chemotherapy. 2011;55(7):3345–3356. PubMed PMID: 21502617.

Palmer K. L., Kos V. N., Gilmore M. S. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. Current Opinion in Microbiology. 2010;13(5):632–639. PubMed PMID: 20837397.

Panesso D., Abadía-Patiño L., Vanegas N., Reynolds P. E., Courvalin P., Arias C. A. Transcriptional analysis of the VanC cluster from Enterococcus gallinarum strains with constitutive and inducible vancomycin resistance. Antimicrobial Agents and Chemotherapy. 2005;49(3):1060–1066. PubMed PMID: 15728903.

Panesso D., Reyes J., Rincón S., Díaz L., Galloway-Peña J., Zurita J., et al. Molecular epidemiology of vancomycin-resistant Enterococcus faecium: a prospective, multicenter study in South American hospitals. Journal of Clinical Microbiology. 2010;48(5):1562–1569. PubMed PMID: 20220167.

Patel R., Allen S. L., Manahan J. M., Wright A. J., Krom R. A., Wiesner R. H., et al. Natural history of vancomycin-resistant enterococcal colonization in liver and kidney transplant recipients. Liver Transplantation. 2001;7(1):27–31. PubMed PMID: 11150418.

Patiño L. A., Chippaux M., Courvalin P., Périchon B. Silencing of glycopeptide resistance in Enterococcus faecalis BM4405 by novobiocin. Antimicrobial Agents and Chemotherapy. 2005;49(4):1419–1425. PubMed PMID: 15793121.

Paulsen I. T., Banerjei L., Myers G. S., Nelson K. E., Seshadri R., Read T. D., et al. Role of mobile DNA in the evolution of vancomycin-resistant Enterococcus faecalis. Science. 2003;299(5615):2071–2074. PubMed PMID: 12663927.

Polidori M., Nuccorini A., Tascini C., Gemigani G., Iapoce R., Leonildi A., et al. Vancomycin-resistant Enterococcus faecium (VRE) bacteremia in infective endocarditis successfully treated with combination daptomycin and tigecycline. Journal of Chemotherapy. 2011;23(4):240–241. PubMed PMID: 21803704.

Prystowsky J., Siddiqui F., Chosay J., Shinabarger D. L., Millichap J., Peterson L. R., et al. Resistance to linezolid: characterization of mutations in rRNA and comparison of their occurrences in vancomycin-resistant enterococci. Antimicrobial Agents and Chemotherapy. 2001;45(7):2154–2156. PubMed PMID: 11408243.

Pujol C., Ehrlich S. D., Jannière L. The promiscuous plasmids pIP501 and pAMß1 from gram-positive bacteria encode complementary resolution functions. Plasmid. 1994;31:100–105. PubMed PMID: 8171120.

Qu T. T., Yang Q., Shen P., Wei Z. Q., Yu Y. S. Novel Vancomycin-Resistance Transposon, Plasmid Replicon Types, and Virulence Factors of Vancomycin-Resistant Enterococci in Zhejiang, China. Microbial Drug Resistance. 2012;18(2):183–188. PubMed PMID: 22257297.

Quintiliani R. Jr, Courvalin P. Characterization of Tn1547, a composite transposon flanked by the IS16 and IS256-like elements, that confers vancomycin resistance in Enterococcus faecalis BM4281. Gene. 1996;172(1):1–8. PubMed PMID: 8654967.

Rand K. H., Houck H. J., Silverman J. A. Daptomycin-reversible rifampicin resistance in vancomycin-resistant Enterococcus faecium. Journal of Antimicrobial Chemotherapy. 2007;59(5):1017–1020. PubMed PMID: 17369277.

Reynolds P. E., Courvalin P. Vancomycin resistance in enterococci due to synthesis of precursors terminating in D-alanyl-D-serine. Antimicrobial Agents and Chemotherapy. 2005;49(1):21–25. PubMed PMID: 15616270.

Rice L. B. Beta -lactam antibiotics and gastrointestinal colonization with vancomycin-resistant enterococci. The Journal of Infectious Diseases. 2005;24(12):804–814. PubMed PMID: 14999616.

Rice L. B. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. The Journal of Infectious Diseases. 2008;197(8):1079–1081. PubMed PMID: 18419525.

Rice L. B., Carias L. L. Transfer of Tn5385, a composite, multiresistance element from Enterococcus faecalis. Journal of Bacteriology. 1998;180(3):714–721. PubMed PMID: 9457879.

Rice L. B., Marshall S. H. Evidence of incorporation of the chromosomal ß-lactamase gene of Enterococcus faecalis CH19 into a transposon derived from staphylococci. Antimicrobial Agents and Chemotherapy. 1992;36(9):1843–1846. PubMed PMID: 1329630.

Rice L. B., Bellais S., Carias L. L., Hutton-Thomas R., Bonomo R. A., Caspers P., et al. Impact of specific Pbp5 mutations on expression of beta-lactam resistance in Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2004;48(8):3028–3032. PubMed PMID: 15273117.

Rice L. B., Calderwood S. B., Eliopoulos G. M., Farber B. F., Karchmer A. W. Enterococcal endocarditis: a comparison of native and prosthetic valve disease. Reviews of Infectious Diseases. 1991;13(1):1–7. PubMed PMID: 2017607.

Rice L. B., Carias L. L., Marshall S. H. Tn5384, a composite enterococcal mobile element conferring resistance to erythromycin and gentamicin whose ends are directly repeated copies of IS256. Antimicrobial Agents and Chemotherapy. 1995;39(5):1147–1153. PubMed PMID: 7625803.

Rice L. B., Carias L. L., Hutton-Thomas R., Rudin S. Interaction of related Tn916-like transposons: analysis of excision events promoted by Tn916 and Tn5386 integrases. Journal of Bacteriology. 2007;189(10):3909–3917. PubMed PMID: 17322310.

Rice L. B., Carias L. L., Marshall S., Rudin S. D., Hutton-Thomas R. Tn5386, a novel Tn916-like mobile element in Enterococcus faecium D344R that interacts with Tn916 to yield a large genomic deletion. Journal of Bacteriology. 2005;187(19):6668–6677. PubMed PMID: 16166528.

Rice L. B., Carias L. L., Rudin S., Hutton R., Marshall S., Hassan M., et al. Role of class A penicillin-binding proteins in the expression of beta-lactam resistance in Enterococcus faecium. Journal of Bacteriology. 2009;191(11):3649–3656. PubMed PMID: 19304851.

Rice L. B., Carias L. L., Rudin S., Lakticová V., Wood A., Hutton-Thomas R. Enterococcus faecium low-affinity pbp5 is a transferable determinant. Antimicrobial Agents and Chemotherapy. 2005;49(12):5007–5012. PubMed PMID: 16304165.

Rice L. B., Lakticová V., Carias L. L., Rudin S., Hutton R., Marshall S. H. Transferable capacity for gastrointestinal colonization in Enterococcus faecium in a mouse model. The Journal of Infectious Diseases. 2009;199(3):342–349. PubMed PMID: 19049434.

Rowland S. J., Dyke K. G. Tn552, a novel transposable element from Staphylococcus aureus. Molecular Microbiology. 1990;4(6):961–975. PubMed PMID: 2170815.

Sacco E., Hugonnet J. E., Josseaume N., Cremniter J., Dubost L., Marie A., et al. Activation of the L,D-transpeptidation peptidoglycan cross-linking pathway by a metallo-D,D-carboxypeptidase in Enterococcus faecium. Molecular Microbiology. 2010;75(4):874–885. PubMed PMID: 20025663.

Safdar A., Bryan C. S., Stinson S., Saunders D. E. Prosthetic valve endocarditis due to vancomycin-resistant Enterococcus faecium: treatment with chloramphenicol plus minocycline. Clinical Infectious Diseases. 2002;34(11):E61–E63. PubMed PMID: 12015709.

Sahm D. F., Kissinger J., Gilmore M. S., Murray P. R., Mulder R., Solliday J., et al. In vitro susceptibility studies of vancomycin-resistant Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 1989;33(9):1588–1591. PubMed PMID: 2554802.

Sakoulas G., Bayer A. S., Pogliano J., Tsuji B. T., Yang S. J., Mishra N. N., et al. Ampicillin enhances daptomycin- and cationic host defense peptide-mediated killing of ampicillin- and vancomycin-resistant Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2012;56(2):838–844. PubMed PMID: 22123698.

Saleh-Mghir A., Lefort A., Petegnief Y., Dautrey S., Vallois J.-M., Le Guludec D., et al. Activity and diffusion of LY333328 in experimental endocarditis due to vancomycin-resistant Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 1999;43(1):115–120. PubMed PMID: 9869575.

Sarti M., Campanile F., Sabia C., Santagati M., Gargiulo R., Stefani S. Polyclonal diffusion of beta-lactamase-producing Enterococcus faecium. Journal of Clinical Microbiology. 2012;50(1):169–172. PubMed PMID: 22075588.

Scapellato P. G., Ormazabal C., Scapellato J. L., Bottaro E. G. Meningitis due to vancomycin-resistant Enterococcus faecium successfully treated with combined intravenous and intraventricular chloramphenicol. Journal of Clinical Microbiology. 2005;43(7):3578–3579. PubMed PMID: 16000513.

Schutt A. C., Bohm N. M. Multidrug-resistant Enterococcus faecium endocarditis treated with combination tigecycline and high-dose daptomycin. The Annals of Pharmacotherapy. 2009;43(12):2108–2112. PubMed PMID: 19887592.

Segarra R. A., Booth M. C., Morales D. A., Huycke M. M., Gilmore M. S. Molecular characterization of the Enterococcus faecalis cytolysin activator. Infection and Immunity. 1991;59(4):1239–1246. PubMed PMID: 1900808.

Shah I. M., Laaberki M. H., Popham D. L., Dworkin J. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. Cell. 2008;135(3):486–496. PubMed PMID: 18984160.

Shaw J. H., Clewell D. B. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B resistance transposon Tn917 in Streptococcus faecalis. Journal of Bacteriology. 1985;164(2):782–796. PubMed PMID: 2997130.

Shepard B. D., Gilmore M. S. Antibiotic-resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. Microbes and Infection. 2002;4(2):215–224. PubMed PMID: 11880055.

Shlaes D. M., Bouvet A., Devine C., Shales J. H., al-Obeid S., Williamson R. Inducible, transferable resistance to vancomycin in Enterococcus faecalis A256. Antimicrobial Agents and Chemotherapy. 1989;33(2):198–203. PubMed PMID: 2497704.

Silverman J. A., Perlmutter N. G., Shapiro H. M. Correlation of daptomycin bactericidal activity and membrane depolarization in Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2003;47(8):2538–2544. PubMed PMID: 12878516.

Singh K. V., Murray B. E. Differences in the Enterococcus faecalis lsa locus that influence susceptibility to quinupristin-dalfopristin and clindamycin. Antimicrobial Agents and Chemotherapy. 2005;49(1):32–39. PubMed PMID: 15616272.

Singh K. V., Murray B. E. Efficacy of Ceftobiprole Medocaril against Enterococcus faecalis in a Murine Urinary Tract infection. Antimicrobial Agents and Chemotherapy. 2012;56(6):3457–3460. PubMed PMID: 22450988.

Singh K. V., Weinstock G. M., Murray B. E. An Enterococcus faecalis ABC homologue (Lsa) is required for the resistance of this species to clindamycin and quinupristin-dalfopristin. Antimicrobial Agents and Chemotherapy. 2002;46(6):1845–1850. PubMed PMID: 12019099.

Squeglia F., Marchetti R., Ruggiero A., Lanzetta R., Marasco D., Dworkin J., et al. Chemical basis of peptidoglycan discrimination by PrkC, a key kinase involved in bacterial resuscitation from dormancy. Journal of the American Chemical Society. 2011;133(51):20676–20679. PubMed PMID: 22111897.

Stevens M. P., Edmond M. B. Endocarditis due to vancomycin-resistant enterococci: case report and review of the literature. Clinical Infectious Diseases. 2005;41(8):1134–1142. PubMed PMID: 16163631.

Sullivan A., Edlund C., Nord C. E. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet. 2001;1(2):101–114. PubMed PMID: 11871461.

Swartley J. S., McAllister C. F., Hajjeh R. A., Heinrich D. W., Stephens D. S. Deletions of Tn916-like transposons are implicated in tetM-mediated resistance in pathogenic Neisseria. Molecular Microbiology. 1993;10(2):299–310. PubMed PMID: 7934821.

Tascini C., Doria R., Leonildi A., Martinelli C., Menichetti F. Efficacy of the combination ampicillin plus ceftriaxone in the treatment of a case of enterococcal endocarditis due to Enterococcus faecalis highly resistant to gentamicin: efficacy of the "ex vivo" synergism method. Journal of Chemotherapy. 2004;16(4):400–403. PubMed PMID: 15332717.

Thal L. A., Silverman J., Donabedian S., Zervos M. J. The effect of Tn916 insertions on contour-clamped homogeneous electrophoresis patterns of Enterococcus faecalis. Journal of Clinical Microbiology. 1997;35(4):969–972. PubMed PMID: 9157164.

Thauvin C., Eliopoulos G. M., Willey S., Wennersten C., Zervos M. J. Continuous infusion ampicillin therapy of enterococcal endocarditis in rats. Antimicrobial Agents and Chemotherapy. 1987;31:139–143. PubMed PMID: 3105445.

Tomayko J. F., Zscheck K. K., Singh K. V., Murray B. E. Comparison of the ß-lactamase gene cluster in clonally distinct strains of Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 1996;40(5):1170–1174. PubMed PMID: 8723460.

Tomich P. K., An F. Y., Clewell D. B. Properties of erythromycin-inducible transposon Tn917 in Streptococcus faecalis. Journal of Bacteriology. 1980;141(3):1366–1734. PubMed PMID: 6245068.

Tripodi M. F., Locatelli A., Adinolfi L. E., Andreana A., Utili R. Successful treatment with ampicillin and fluoroquinolones of human endocarditis due to high-level gentamicin-resistant enterococci. European Journal of Clinical Microbiology & Infectious Diseases. 1998;17(10):734–736. PubMed PMID: 9865990.

Twilla J. D., Finch C. K., Usery J. B., Gelfand M. S., Hudson J. Q., Broyles J. E. Vancomycin-resistant Enterococcus bacteremia: an evaluation of treatment with linezolid or daptomycin. Journal of Hospital Medicine. 2012;7(3):243–248. PubMed PMID: 22076962.

Ubeda C., Taur Y., Jeng R. R., Equinda M. J., Son T., Samstein M., et al. Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. The Journal of Clinical Investigation. 2010;120(12):4332–4341. PubMed PMID: 21099116.

Van Tyne D., Martin M. J., Gilmore M. S. Structure, Function, and Biology of the Enterococcus faecalis Cytolysin. Toxins. 2013;5(5):895–911. PubMed PMID: 23628786.

Vergis E. N., Hayden M. K., Chow J. W., Snydman D. R., Zervos M. J., Linden P. K., et al. Determinants of vancomycin resistance and mortality rates in enterococcal bacteremia. a prospective multicenter study. Annals of Internal Medicine. 2001;135(7):484–492. PubMed PMID: 11578151.

Vesić D., Kristich C. J. MurAA Is Required for Intrinsic Cephalosporin Resistance of Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2012;56(5):2443–2451. PubMed PMID: 22290954.

Vogler W. R., Dorney E. R., Bridges H. A. Bacterial endocarditis. A review of 148 cases. The American Journal of Medicine. 1962;32(6):910–921. PubMed PMID: 13926470.

Webster D. P., Griffiths S., Bowler I. C. Failure of linezolid therapy for post-neurosurgical meningitis due to Enterococcus faecium. Journal of Antimicrobial Chemotherapy. 2009;63:622–623. PubMed PMID: 19126668.

Wegener H. C., Madsen M., Nielsen N., Aarestrup F. M. Isolation of vancomycin resistant Enterococcus faecium from food. International Journal of Food Microbiology. 1997;35(1):57–66. PubMed PMID: 9081226.

Wehrli W., Knüsel F., Schmid K., Staehelin M. Interaction of rifamycin with bacterial RNA polymerase. Proceedings of the National Academy of Sciences. 1968;61(2):667–673. PubMed PMID: 4879400.

Welton L. A., Thal L. A., Perri M. B., Donabedian S., McMahon J., Chow J. W., et al. Antimicrobial resistance in enterococci isolated from Turkey flocks fed virginiamycin. Antimicrobial Agents and Chemotherapy. 1998;42(3):705–708. PubMed PMID: 9517958.

Werner G., Fleige C., Ewert B., Laverde-Gomez J. A., Klare I., Witte W. High-level ciprofloxacin resistance among hospital-adapted Enterococcus faecium (CC17). International Journal of Antimicrobial Agents. 2010;35(2):119–125. PubMed PMID: 20006472.

Werner G., Freitas A. R., Coque T. M., Sollid J. E., Lester C., Hammerum A. M., et al. Host range of enterococcal vanA plasmids among Gram-positive intestinal bacteria. Journal of Antimicrobial Chemotherapy. 2011;66(2):273–282. PubMed PMID: 21131318.

Willem R. J., Top J., van Santen M., Robinson D. A., Coque T. M., Baquero F., et al. Global spread of vancomycin-resistant Enterococcus faecium from distinct nosocomial genetic complex. Emerging Infectious Diseases. 2005;11(6):821–828. PubMed PMID: 15963275.

Williamson R., Al-Obeid S., Shlaes J. H., Goldstein F. W., Shlaes D. M. Inducible resistance to vancomycin in Enterococcus faecium D366. Journal of Infectious Diseases. 1989;159(6):1095–1104. PubMed PMID: 2723455.

Wu S. W., de Lencastre H., Tomasz A. The Staphylococcus aureus transposon Tn551: complete nucleotide sequence and transcriptional analysis of the expression of the erythromycin resistance gene. Microbial Drug Resistance. 1999;5(1):1–7. PubMed PMID: 10332716.

Xu X., Lin D., Yan G., Ye X., Wu S., Guo Y., et al. VanM, a new glycopeptide resistance gene cluster found in Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2010;54(11):4643–4647. PubMed PMID: 20733041.

Yeats C., Finn R. D., Bateman A. The PASTA domain: a beta-lactam-binding domain. Trends in Biochemical Sciences. 2002;27(9):438. PubMed PMID: 12217513.

Zervos M. J., Schaberg D. R. Reversal of in vitro susceptibility of enterococci to trimethoprim-sulfamethoxazole by folinic acid. Antimicrobial Agents and Chemotherapy. 1985;28(3):446–448. PubMed PMID: 3935044.

Zheng B., Tomita H., Inoue T., Ike Y. Isolation of VanB-type Enterococcus faecalis strains from nosocomial infections: first report of the isolation and identification of the pheromone-responsive plasmids pMG2200, Encoding VanB-type vancomycin resistance and a Bac41-type bacteriocin... Antimicrobial Agents and Chemotherapy. 2009;53(2):735–747. PubMed PMID: 19029325.

Zhu W., Murray P. R., Huskins W. C., Jernigan J. A., McDonald L. C., Clark N. C., et al. Dissemination of an Enterococcus Inc18-Like vanA plasmid associated with vancomycin-resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2010;54(10):4314–4320. PubMed PMID: 20660665.

Zimmermann R. A., Moellering R. C. Jr, Weinberg A. N. Mechanism of resistance to antibiotic synergism in enterococci. Journal of Bacteriology. 1971;105:873–879. PubMed PMID: 4994038.

License