Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce?

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Twenty-five years ago, isolation of vancomycin-resistant Enterococcus faecium (VREm) was reported both in the UK and in France. Since then, VREm has spread worldwide in hospitals. Hospital outbreaks appeared to be related to the evolution since the end of 1980s of a subpopulation of E. faecium highly resistant to ampicillin and fluoroquinolones (the so-called clonal complex CC17) that later acquired resistance to vancomycin. CC17 isolates are presumably better adapted than other E. faecium isolates to the constraints of the hospital environment and most contain mobile genetic elements, phage genes, genes encoding membrane proteins, regulatory genes, a putative pathogenicity island and megaplasmids. Colonization and persistence are major features of VREm. Inherent characteristics of E. faecium including a remarkable genome plasticity, in part due to acquisition of IS elements, in particular IS16, have facilitated niche adaptation of this distinct E. faecium subpopulation that is multiply resistant to antibiotics. Quinupristin/dalfopristin and linezolid are licensed for the treatment of VREm infections, with linezolid often used as a first-line treatment. However, the emergence of plasmid-mediated resistance to linezolid by production of a Cfr methyltransferase in Enterococcus faecalis is worrying. Daptomycin has not been extensively evaluated for the treatment of VREm infections and resistant mutants have been selected under daptomycin therapy. Although control of VRE is challenging, a laissez-faire policy would result in an increased number of infections and would create an irreversible situation. Although so far unsuccessful, dissemination of glycopeptide-resistant Staphylococcus aureus with van genes acquired from resistant enterococci cannot be ruled out.

Keywords: VRE, vancomycin, nosocomial infection, clonality, antibiotic resistance, MDR

Introduction

Twenty-five years ago, isolation of vancomycin-resistant enterococci (VRE) was reported both in the UK and in France.1,2 These reports came as a nasty surprise since vancomycin had been used for more than 30 years without any report of resistance, leading the medical and scientific community to believe that resistance to glycopeptides was unlikely to emerge. Although initially the clinical significance of these reports was not widely appreciated, their impact subsequently became apparent with the rapid spread of VRE in US hospitals in the 1990s,3 in European hospitals in the 2000s,4 and eventually worldwide.5

VRE brought with it several other surprises. First, extensive research work deciphered one of the most complex mechanisms of resistance described so far.6 Then, epidemiological studies revealed the role of avoparcin, a glycopeptide used as a growth promoter in food animals, in the selection of animal VRE isolates that could be further transmitted to humans via the food chain.5,7 During the years 1995–2005, this led to the banning of all antibiotics used as growth promoters in the European Union.7 In addition, the in vivo transfer of mobile genetic elements bearing vancomycin resistance genes to methicillin-resistant Staphylococcus aureus (MRSA) was anticipated on the basis of in vitro transfer.8 Although this unfortunate event has occurred, these organisms remain uncommon, and the nightmare scenario originally envisaged did not happen.9 Despite considerable research, the reasons for the emergence and rapid spread of VRE remain obscure. Also, our capacity to control the spread of VRE is still a matter of debate.10 This review updates the evolution of VRE epidemiology 25 years after the first reports and describes how this species has evolved to cope with a constantly changing environment. It also highlights the challenges raised by these multidrug-resistant (MDR) bacteria.

The puzzling mechanism of resistance to vancomycin

The synthesis of peptidoglycan, the major component of bacterial cell walls, requires several steps (Figure 1).11–13 In the cytoplasm, a racemase converts L-alanine to D-alanine (D-Ala), and then two molecules of D-Ala are joined by a ligase...
creating the dipeptide D-Ala-D-Ala, which forms uracil diphosphate-N-acetylmuramyl-pentapeptide through several enzymatic reactions. This precursor is then bound to the undecaprenol lipid carrier, which allows its translocation to the outer surface of the cytoplasmic membrane. Finally, N-acetylmuramyl-pentapeptide is incorporated into nascent peptidoglycan by transglycosylation with the formation of cross-bridges between the peptide side chains by transpeptidation. Glycopeptides do not enter the bacterial cytoplasm, but are bound to the external surface of the cytoplasmic membrane, where they inhibit the synthesis of peptidoglycan. They bind with high affinity to the D-Ala-D-Ala C terminus of the pentapeptide precursors by five hydrogen bonds and, because of their high molecular weight (approximately 1500–2000 Da), they block by steric hindrance the transglycosylation linkage of late precursors to the nascent peptidoglycan chain and prevent subsequent cross-linking by transpeptidation. Resistance to glycopeptides is due to the production of low-affinity pentapeptide precursors ending either in D-lactate (D-Lac) or D-serine (D-Ser) and the elimination of the normally produced high-affinity precursors (ending in D-Ala-D-Ala). This target modification results from the cooperation of several enzymes required for reprogramming peptidoglycan synthesis that are encoded by genes organized in an operon. The D-Ala to D-Lac change in the terminus of the precursor leads to the abolition of an essential hydrogen bond that results in turn in a 1000-fold decrease in affinity of vancomycin for precursors ending in D-Ala-D-Ala (which results in high-level resistance to vancomycin). In contrast, the change from D-Ala to D-Ser leads only to a 7-fold reduction in affinity and thus to a lower level of resistance to vancomycin.

So far, eight types of acquired resistance to glycopeptides have been reported on the basis of phenotypic and genotypic criteria (VanA, VanB, VanD, VanE, VanG, VanL, VanM, and VanN) with one other type of resistance (VanC) being an intrinsic

![Figure 1. Schematic representation of peptidoglycan biosynthesis and action mechanism of glycopeptides. Peptidoglycan biosynthesis occurs in three steps: (i) a first step in the cytoplasm (C) with the formation of UDP-N-acetyl-muramyl-pentapeptides from UDP-N-acetyl-muramyl-tripeptides and D-Ala-D-Ala dipeptides; (ii) a translocation step through the cytoplasmic membrane (M) of pentapeptide precursors as lipid-linked intermediates and (iii) a final step consisting of the incorporation of precursors to the nascent peptidoglycan by transglycosylation and the formation of cross-links by D,D-transpeptidation into the cell wall (W). Glycopeptides bind to the D-Ala-D-Ala extremity of pentapeptide precursors and thus inhibit the final steps of peptidoglycan biosynthesis (i.e. transglycosylation and D,D-transpeptidation).]
characteristic of Enterococcus gallinarum and Enterococcus casseliflavus (Table 1).6,12,15–18 VanA and VanB are the types most frequently detected in enterococci and have occasionally been detected in coryneform bacteria and streptococci.19 The VanA operon has also, albeit rarely, been detected in S. aureus.9 VanA-positive isolates are inducibly resistant to high levels of vancomycin and teicoplanin (MIC >64 mg/L), whereas VanB-positive isolates display various levels of inducible resistance to vancomycin (MICs generally equal to 16–32 mg/L), but remain susceptible to teicoplanin, as it is not an inducer. The VanA operon is usually borne by a Tn3-type transposon (Tn1546) that is composed of five genes involved in resistance to glycopeptides (vanHAXYZ) and two regulatory genes (vanRS).6 The vanA gene encodes a ligase that catalyses the formation of a D-Ala-D-Lac depsipeptide. However, the vanA gene alone cannot confer resistance since the D-Lac substrate is rare in nature and needs to be synthesized. This is the role of the dehydrogenase encoded by vanH that reduces pyruvate, commonly found in nature, to D-Lac, thus providing the substrate for the ligase. In the following steps, this depsipeptide is finally incorporated as a pentadepsipeptide form into the growing peptidoglycan. However, resistance is still not expressed since the synthesis of wild-type precursors persists and allows binding of vancomycin and subsequent disruption of the cell wall. Elimination of these ‘susceptible’ precursors is the task of the vanX gene that encodes a d,L-dipeptidase able to hydrolyse the dipeptide d-Ala-d-Ala formed by the natural ligase (Ddl). In addition, the vanY genes encoding a d,D-carboxypeptidase eliminates the terminal d-Ala of precursors in case of incomplete elimination of the dipeptide by VanX. A similar mechanism operates with the VanB type of resistance, where the resistance operon is borne by the Tn1547 transposon.

The d-Ala-d-Ser-type gene clusters are acquired, except for VanC, which is intrinsic to E. gallinarum and E. casseliflavus.20 VanE, VanG and VanL are rare and have so far been detected only in the chromosome of Enterococcus faecalis, while VanN has only been found in a single isolate of Enterococcus faecium.8,15,16,18 VanG and VanN are the only transferable d-Ala-d-Ser resistance types characterized so far.16,18 It should be noted that certain strains of E. faecalis and E. faecium (VanA or VanB type) that are not only resistant to vancomycin, but are also dependent on the presence of vancomycin or teicoplanin for their survival, have been isolated in vitro and from patients.21,22 This remarkable trait is related to the lack of a functional natural Ddl ligase due to mutations in the ddl gene. Consequently, in these strains the synthesis of peptidoglycan is entirely dependent on the expression of the resistance operon after induction by a glycopeptide.

The origin of vancomycin resistance in clinical isolates remains uncertain. It may have emanated from glycopeptide-producing organisms such as Streptomyces tsukaharaensis and Amycolatopsis orientalis that possess van operons distantly related to the enterococcal van operons.23 In Paenibacillus popilliae, a vancomycin-resistant biopesticide, an operon showing higher homology to the sequence of the enterococcal vanA operon has been reported and may more likely have been a precursor to, or have had a common ancestral origin with, the VanA operon.24 The origin of vancomycin resistance is undoubtedly ancient, since vanHAXY-related sequences have been recovered from 30 000 year old samples obtained from permafrost collected in the Yukon (Canada).25

Table 1. Types of resistance to glycopeptides in enterococci6,16 –18

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R, high level of resistance (MIC >16 mg/L); R, low level of resistance (MIC = 8–16 mg/L); S, susceptible; A, E. faecium; B, E. faecalis; G, E. gallinarum, D, E. casseliflavus; I, inducible; C, constitutive; Chr, chromosome.

aAlso other enterococcus species.
The early story of VRE and unexpected further consequences

The first VRE isolates reported in 1988 in the UK and in France belonged to the species *E. faecium* (VREM).1,2 From an early stage it appeared that the prevalence of VREM carriage in the community was high in Europe, with, for example, a carriage rate of 28% being seen in a population of students from an engineering school in Belgium, in contrast to the rarity of hospital outbreaks.26 The role of avoparcin, a glycopeptide antibiotic used as a growth promoter in food animals, was rapidly suspected as a selective agent for resistant strains that could be further transmitted to humans via the food chain.27 There is now a considerable amount of evidence that vancomycin-resistant organisms have been selected in animals by avoparcin.28 Moreover, transmission to humans through the food chain, while not categorically proven, seems very likely. Both human commensal and animal strains of VREM remained generally susceptible to ampicillin.29

These observations had major consequences, as they led to reconsideration of the use of antibiotics for non-therapeutic purposes in animals (including growth promotion) in Europe. Despite the fact that animal VRE could spread in humans and might cause serious human infections, the European Union (EU) decided in 1995–1997 to ban the use of avoparcin. This decision was based on the precautionary principle that applies when scientific evidence is inconclusive but scientific evaluation indicates that there are reasonable grounds for concern regarding human health impairment. This decision, followed by a ban on all the other antimicrobials used as growth promoters in the 2000s, was the forerunner of many controversies generated by the lack of thorough and comprehensive risk analyses.7,30 These debates and controversies, which were also experienced with other food safety issues where there were conflicts between precautionary actions taken to safeguard public health and international trade (e.g. beef hormone, bovine spongiform encephalopathy and genetically modified organisms), have attracted intense and sustained attention.7,31 So far, however, the European ban has not been followed by other nations.

The ban on avoparcin resulted in a statistically significant decline in VRE isolated from chickens in Denmark.32 Similar observations were made in several countries, including a reduction in the isolation of VRE from chickens and pigs in France.13 However, the decline was slow and the resistance determinants still persist years after a complete cessation of glycopeptide consumption by farm animals and despite a high biological cost of vancomycin resistance.34 Therefore an animal reservoir of resistance genes may still be present and may potentially persist for years to come.

The situation in European hospitals was initially reassuring, with only a few outbreaks in high-risk units such as intensive care, transplant, haematology and renal units. However, a few years later, in the 2000s, while the carriage of VRE by animals steeply declined, some EU countries experienced an increase in VRE and large hospital outbreaks. The European Antimicrobial Resistance Surveillance System (EARSS, now known as EARSNet) revealed that the proportion of glycopeptide-resistant *E. faecium* isolated from blood has dramatically increased since 2000 in several European countries (e.g. Ireland, Germany and Greece), leading to a situation resembling that of the USA, described below.6 Moreover, the strains differed from those isolated from animals, in particular in that they were highly resistant to ampicillin.

In the USA, where avoparcin has never been legally available for use in animals and oral and intravenous vancomycin was massively used for human diseases, the spread of VREM in hospitals was reported as early as the end of 1989 in New York City.3 The proportion of VRE (all enterococcal species included) in US hospitals rapidly increased both inside and outside intensive care units (ICUs), reaching around 28% of enterococcal isolates in 1993.35 Initially about 70% of isolates displayed the VanA phenotype and about 25% the VanB phenotype. Most were *E. faecium* that exhibited high-level resistance to ampicillin and aminoglycosides, leaving few options for treatment. It is not anecdotal to mention that this spread was observed in the context of the previous emergence and spread of *E. faecium* isolates highly resistant to ampicillin.36 In contrast, VRE has not been found in community-based volunteers in the USA, in American food-producing animals or in pet animals, although only a few epidemiological surveys have been conducted.37

Where are we with VRE?

As already mentioned, the vast majority of VRE belongs to the species *E. faecium*. The prevalence in Europe of VREM, as reported in 2008–10, varied from high to low, depending on the country.38 In the EARSS report, the descending prevalence gradient from southern to northern Europe classically observed for MRSA and *E. coli* resistant to third-generation cephalosporins was not observed for VREM (Figure 2).4 The proportion of VREM isolated from blood cultures was <5% in France, Italy, Spain and some northern countries, comprised between 5% and 10% in Germany and Poland, and comprised between 10% and 25% in Greece, Portugal and the UK.

A recent study collecting a large number of enterococci from various countries worldwide confirmed the heterogeneity of the situation (Table 2).39 Two-thirds of the study *E. faecium* were from blood and 8% from urine. In the USA, the prevalence of vancomycin resistance is so high in *E. faecium* that it tends to become part of the genetic patrimony of this species, while in Asia, the numbers are not so high, although this may reflect the relatively recent emergence of resistance on this continent. Table 2 also shows that the prevalence of glycopeptide resistance in *E. faecium* is higher than in *E. faecalis*, with the occurrence of resistance in both species being explained by interspecies transfer of mobile resistance determinants. A recent study showed that in the USA, in the last 10 years, the mean prevalence of vancomycin resistance among 7424 bloodstream enterococcal clinical isolates was 27.8%.50 The rate of vancomycin resistance among *E. faecium* increased from 6.1% in 2000 to 80.7% of all bloodstream *E. faecium* infections in 2010, with *E. faecium* now isolated at a rate approaching that of *E. faecalis*.

**E. faecium: born to colonize**

Among microorganisms causing hospital outbreaks, *E. faecium* appears to be more common than the other enterococcal
species, including *E. faecalis*. Interaction with the host resulting in initial colonization followed by persistence rather than lethality is a major feature of *E. faecium*. This would account for its striking capacity to massively colonize healthy carriers and patients, to overcome host defences and to spread, leading to major outbreaks. Generally, heavy stool colonization with numbers of enterococci exceeding $10^8$–$10^{10}$ cfu/g of faeces precedes infection in patients. This interspecies difference of behaviour was demonstrated in a *Galleria mellonella* model of infection, where *E. faecium* could survive and multiply for several days in the larvae, in contrast to *E. faecalis*, which was rapidly lethal. The reasons for this colonizing capacity are not fully understood, although a number of factors involved in the epidemiology of *E. faecium* have recently been reviewed. In addition, an oxidative stress-sensing regulator modulating *E. faecium* opportunistic traits has been recently identified. Oxidative stress serves as an important host/environmental signal that triggers a wide range of responses in microorganisms. This regulator, called AsrR (antibiotic and stress response regulator), belongs to the MarR family, which comprises transcriptional regulators sensing oxidative stress and regulating bacterial responses. The AsrR regulator uses cysteine oxidation to sense hydrogen peroxide, resulting in dissociation of the promoter DNA. Interestingly, AsrR has no homologue in *E. faecalis*. Transcriptome analysis showed that the AsrR regulon comprises 181 genes, including those encoding functionally diverse groups involved in pathogenesis, antibiotic and antimicrobial peptide resistance, oxidative stress and adaptive responses. For instance, expression of the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) Acm, which is an adhesin for collagen and contributes to endocarditis, is controlled in part by this regulator. Based on experiments on AsrR inactivation and complementation, it appears that under oxidative stress conditions, the bacteria display: (i) diminished susceptibility to penicillins, vancomycin and cationic antimicrobial peptides; (ii) increased adhesion, biofilm formation and host colonization; (iii) a mutator phenotype and enhanced DNA transfer frequencies; and, unexpectedly, (iv) decreased susceptibility to oxidative stress both in vitro and in murine macrophages. The first three conditions could promote *E. faecium* host adaptation.

**Insights into niche adaptation of *E. faecium***

Using various molecular typing methods, a distinct number of ecovars have been identified within the species *E. faecium*, allowing differentiation between typical poultry, swine, cattle, human commensal and hospital strain types. In particular, on the basis of multilocus sequence typing (MLST), a subpopulation of highly virulent, hospital-adapted related strain types (ST18, ST17, ST78, ST117, ST192 and ST280) with epidemic potential has been characterized—the so-called clonal complex CC17. Isolates belonging to this complex are characterized by distinct markers such as ampicillin and high-level fluoroquinolone resistance.
The irresistible rise of multiple antibiotic resistance in *E. faecium*

Hospital-adapted *E. faecium* is one of the paradigms of multiple antibiotic resistance. Again, this is another illustration of the genomic plasticity of this species, which enables it to respond to environmental selective pressures, including antimicrobial use. Figure 3 shows the major steps in the acquisition of antimicrobial resistance in enterococci. Apart from the intrinsic antibiotic resistances that are numerous in this bacterial genus, no major problem was identified until the beginning of the 1980s when plasmid-mediated resistance to high levels of gentamicin was characterized in hospital-associated strains of *E. faecalis*. For the first time, epidemiological studies provided evidence that high-level gentamicin-resistant enterococci were a major source of nosocomial infections. This resistance, found later in *E. faecium*, suppresses the bactericidal synergy between cell wall synthesis inhibitors and most aminoglycosides that is essential for the treatment of severe enterococcal infections such as endocarditis. Since staphylococci and enterococci share a similar pool of resistance genes (resistance to macrolides, gentamicin and chloramphenicol), the report in 1983 of penicillinase-producing enterococci was not really a surprise. These strains, which had acquired the staphylococcal plasmid-mediated penicillinase and were also highly resistant to gentamicin, were detected in a few hospitals in the USA, Lebanon and Argentina. Actually, the real surprise has been the absence of dissemination of these potentially dangerous strains for the last 30 years. The low-level expression of penicillin resistance despite its constitutive nature, and possibly some fitness disadvantages for the penicillinase-producing strains as compared with the susceptible population, might explain this unexpected evolution. A few years later the acquisition of high-level resistance to ampicillin by *E. faecium* was a key step in the spread of resistance and the role of this species in nosocomial infections. As mentioned above, although vancomycin resistance was first detected in animal isolates, the genetic determinants were also acquired by strains of the hospital-adapted CC17 group that spread. These strains were resistant to all major antibiotics available in the early 2000s (*β*-lactams, glycopeptides...
and gentamicin). Their epidemiological success was related in part to the fact that a risk factor for acquisition by patients included exposure to almost any of the antibiotics that are commonly used in hospital settings, but in particular cephalosporins, imipenem, fluoroquinolones and anti-anaerobes.58 Fortunately the newer antibiotics linezolid and daptomycin remain generally active (MIC₉₀ at 2 and 1 mg/L, respectively).59 However, linezolid- and daptomycin-resistant strains and a few limited outbreaks of linezolid-resistant isolates have been reported.60,61

Multiple antibiotic resistance combined with the colonization ability of CC17-type E. faecium account for the progressive decrease of the ratio of E. faecalis to E. faecium.62 The ratio, which was about 85%–90% in the 1990s, has decreased to 65% in certain European hospitals.63 In France, the ratio was 73%/27% in 2010, versus 80%/20% in 2005 and 90%/10% at the end of the 1990s.63,64 In the USA, in 2010, the ratio was 1 E. faecium for every 1.8 E. faecalis in blood cultures.40

In a study of enterococcal blood isolates received from clinical microbiology laboratories in 11 Danish counties from 2002 to 2006, a 68% increase in the number of infections was observed.65 The increase was mainly caused by E. faecium CC17 isolates (resistant to ampicillin but not to vancomycin), which tripled, whereas the number of E. faecalis isolates only slightly increased during the same period. This means that the CC17 isolates did not replace the other enterococci but added to them. We are probably not at the end of this evolution, which constitutes a fruitful avenue for VRE.

VRE infection treatment: will we soon hit the wall?

The most serious infections due to VREm occur in severely ill and/or immunocompromised patients. VREm isolates pose major therapeutic problems because most of these strains are also highly resistant to penicillin and ampicillin. In the early 1990s, the first-choice treatment was restricted to chloramphenicol,66 or to tetracyclines or rifampicin for selected strains, although the latter drugs are not particularly useful for the treatment of severe infections.

Since then, only two new antibiotics have been licensed for the treatment of VREm infections, namely quinupristin/dalfopristin in 1999 and linezolid in 2000. The first progress was the development of quinupristin/dalfopristin, an injectable streptomycin.67 However, this drug has several limitations, including the need for central venous administration, metabolic interactions, an adverse-effect profile, a lack of bactericidal activity against VREm isolates, development of resistance and a lack of activity against E. faecalis.68 For these reasons, this antimicrobial is now rarely used in clinical practice. Linezolid, an oxazolidinone antibiotic, is an inhibitor of protein synthesis, available for oral or intravenous administration. Even though it has been shown to be effective in the treatment of various types of VREm infections in multicentre Phase III trials,68 its bacteriostatic mode of action raises concerns regarding the effectiveness of this agent for the treatment of VREm endocarditis. Nonetheless, linezolid has been endorsed as one of the first-line therapeutic options for the treatment of VREm infections, including endocarditis, although the relevant clinical evidence is scarce. Resistance to linezolid is generally due to ribosomal mutations,69 although the worrying description of plasmid-mediated resistance to linezolid (mediated by Cfr methyltransferase) in E. faecalis of animal and human origin strongly suggests that further spread of this resistance in VRE is likely.70

Daptomycin is a lipopeptide antimicrobial that has in vitro bactericidal activity against VRE, with MIC₉₀ values typically of 4 mg/L, versus 0.5 mg/L for S. aureus.71 The CLSI considers that isolates with MICs ≤ 4 mg/L should be considered susceptible to this antibiotic.72 However, EUCAST has not published daptomycin breakpoints for enterococci, while the European Medicines Agency (EMEA) has not endorsed the use of daptomycin for enterococcal infections due to insufficient evidence of clinical efficacy. Actually, clinical use of daptomycin for VRE infections has not been extensively studied, and there are questions regarding the dosage that should be used for the treatment of patients.73 In a rat model of endocarditis, the activity of daptomycin was similar to that of amoxicillin and superior to that of teicoplanin when administered at 6 mg/kg/24 h.74 In contrast, in a rabbit model of endocarditis, daptomycin was only active at a higher dosage of 12 mg/kg/24 h and was most active when combined with gentamicin.75 Further studies demonstrated synergy against highly resistant VREm when cell-wall active agents were combined with daptomycin. Interestingly, the mechanism of the synergy with ampicillin is original, since it relies, at least in part, on enhancement by ampicillin of activity of both daptomycin and innate host defence peptides against VREm.76

Finally, as mentioned above, although the clinical evidence for the efficiency of daptomycin in the treatment of VREm infections is sparse, retrospective studies have shown a success rate of 87%–90% in bacteraemia due to VREm.77,78

In addition, the few reports of resistance emerging during therapy are worrisome. Recently, whole genome sequences of daptomycin-susceptible and -resistant E. faecalis clinical isolates recovered before and after daptomycin therapy, respectively, showed mutations in a membrane protein, LiaF, that is predicted to be a member of a three-component regulatory system (LiaFSR) involved in the cell envelope stress-sensing response to antibiotics.65 Another mutation was also detected in a gene encoding a protein of the glycerophosphoryl diester phosphodiesterase family with a putative role in metabolism of membrane phospholipids.65 The resistant mutant had alterations in the ultrastructure of the cell membrane and cell wall such that daptomycin was less able to depolarize its cell membrane as compared with the susceptible isolate. In another study, mutants of E. faecalis V583 selected for their resistance to daptomycin displayed mutations in genes encoding cardiolipin synthase, a phospholipid synthesis enzyme.79 Finally, tigecycline has an in vitro bacteriostatic activity against VRE, but its clinical use for serious enterococcal infections has not been evaluated. Therefore there are only a few reliable therapies for VRE currently available, but the newer compounds have yet to be properly evaluated in this indication.

The spread of vanA resistance in S. aureus

The first documented case of vancomycin-resistant S. aureus (VRSA) due to acquisition of the vanA operon occurred in Michigan, USA, in 2002.80 Since then, only a dozen instances of MRSA acquiring resistance to vancomycin have been reported,
mainly in the USA (mostly Michigan), India and Iran.\textsuperscript{9,80–84} However, this might be an underestimate of the actual number of VRSA isolates, as there is anecdotal evidence of isolates that have not been reported in the literature, particularly in certain Asian countries.

Genome sequencing of 12 available VRSA showed that they all contained the enterococcal transposon Tn1546 bearing the vanA operon.\textsuperscript{85} Phylogenetic analysis revealed that VRSA strains shared a common ancestor >50 years ago and had particular features, e.g. a cluster of unique superantigens and lipoproteins to confound host immunity.\textsuperscript{85} Most patients infected with such strains presented with several underlying conditions, including chronic skin ulcers, had received vancomycin and had a history of prior MRSA and VRE co-infection or colonization. Fortunately, but surprisingly, there was no subsequent patient-to-patient dissemination of these microorganisms even though the US isolates belonged to the MLST type ST5, which includes classical MRSA USA100 and USA800 clones.\textsuperscript{9}

Several explanations may combine to account for the relative absence of (or slow) dissemination of VRSA. Importantly, rapid detection by microbiology laboratories and adherence to recommended infection control measures for MDR organisms may have efficiently prevented cross-transmission of the microorganisms. However, some aspects of the physiology and genetics of VRSA are also important to consider. First, at least in one strain, the plasmid bearing the vanA operon was unstable and expression of resistance required a long time for induction.\textsuperscript{86} Also, only a limited number of enterococcal donor plasmids are broad-host-range conjugal plasmids. Inc18-like plasmids that may transfer from enterococci to staphylococci have been identified as bearing the van determinants in S. aureus or in VRE isolates from the same patient.\textsuperscript{87} A recent study showed that this plasmid type is rare in enterococci, particularly in \textit{E. faecium.} Of all vancomycin-resistant \textit{E. faecalis} isolates tested, 6.0\% were positive for the plasmid, compared with 0.6\% for \textit{E. faecium.} In addition, staphylococcal restriction enzymes are barriers for the acquisition of foreign genes, in particular type III-like restriction endonucleases.\textsuperscript{88} Only a few MRSA isolates are deficient in this system, and hypothetically it is only these that may easily acquire vancomycin resistance genes from enterococci. However, restriction systems were recently shown to be intact in most VRSA strains.\textsuperscript{85} In the absence of induction by vancomycin, tight regulation of resistance expression drastically reduces the biological cost associated with vancomycin resistance in enterococci, favouring their dissemination.\textsuperscript{89} However, the situation is different in \textit{S. aureus,} as regulation is not so efficient and the basal level of expression of the van genes in the absence of induction results in a slight fitness burden.\textsuperscript{90}

Finally, combinations of vancomycin (or teicoplanin) and oxacillin/nafcillin have a strong synergistic effect at concentrations that are achievable in therapy against methicillin-resistant VRSA both in vitro and in an animal model despite resistance of the strains to both drug classes when administered separately.\textsuperscript{91} The common use of combinations of vancomycin and \(\beta\)-lactams in ICUs as empirical treatment may thus limit dissemination of VRSA in hospital settings. The mechanism of synergy may be explained as follows.\textsuperscript{86} Methicillin resistance is due to the synthesis of an additional low-affinity penicillin-binding protein (PBP2a) encoded by the mecA gene and PBP2a is a transpeptidase that remains active in the presence of \(\beta\)-lactams. In the presence of vancomycin, bacteria synthesize modified peptidoglycan precursors ending in \textit{o-Ala-o-Lac} that are not substrates for PBP2a in the transpeptidation step but can be processed by active PBP2. If oxacillin is combined with vancomycin, the transpeptidase activity of PBP2 is suppressed by the \(\beta\)-lactam and synthesis of peptidoglycan cannot proceed, resulting in death of the bacteria.

### Control of VRE: laissez-faire or a battle to win?

Control of VRE is considered highly challenging for a number of reasons.\textsuperscript{10} First, the reservoir of VRE is wide and occult, unsuspected faecal carrier patients being a major reservoir. Generally, for one hospitalized patient with an infection caused by VRE, from 2 to 10 contact patients are faecal carriers. Since unsuspected carriers are a major source for dissemination, the role of VRE importation from repellants and travellers hospitalized in foreign countries with high VRE prevalence should be considered in countries with a low VRE prevalence.\textsuperscript{92}

A major problem for definitive control of VRE is the magnitude of the reservoir, which extends far beyond the human gastrointestinal tract. Numerous animals, such as ducks, chicken, pigs, horses, cows, goats and pets are carriers.\textsuperscript{5,27} VREM have also been found in urban and hospital wastewaters, various food products, meats, vegetables and cheese.\textsuperscript{25,93–96} Remarkably, houseflies may contaminate meat with approximately \(10^4\,\text{cfu}\) of enterococci within 30 min.\textsuperscript{97} In the wild, a variety of animals, including badgers, wild boars, wild rabbits, woodmice and even a polar gull have been reported as VRE carriers.\textsuperscript{98–101} The polyclonality of animal strains suggests the spread of mobile genetic elements, e.g. Tn1546-type elements, among different clones of enterococci.

Another consideration is that van genes are not confined to enterococci. Anaerobes are considered as a major reservoir for these genes, as vanB genes and the Tn1549-like element have been detected in \textit{Clostridium} spp., \textit{Eggerthella lenta} and \textit{Ruminococcus} spp., while vanD and vanG were found in \textit{Ruminococcus} spp.\textsuperscript{102–104} Transfer of the Tn1549-like element (vanB) from \textit{Clostridium symbiosum} to \textit{E. faecium} and \textit{E. faecalis} in the digestive tract of gnotobiotic mice has been reported, demonstrating in vivo exchanges of vancomycin resistance genes between phylogenetically remote species.\textsuperscript{105} The presence of van genes in non-enterococcal intestinal bacteria explains the poor specificity of PCR aimed at detecting van genes in patients’ stools, especially for vanB.\textsuperscript{106,107} In addition, CC17 \textit{E. faecium} are now permanent residents of hospital settings and will probably be difficult to eradicate.

When weighing the cumbersome and aggressive measures that should be implemented to effectively control VRE, the huge reservoir of resistance genes and the failure of control in several countries (e.g. in the USA), coupled with the weak pathogenicity of VREM, have led some people to think that it is not even worth doing. However, the clinical importance and burden of VRE should not be underestimated. A meta-analysis of published studies has concluded that VRE bloodstream infections are associated with higher recurrence rates, mortality and excess costs than bloodstream infections with vancomycin-susceptible enterococci, including multiple studies adjusting for the severity of illness.\textsuperscript{108} A laissez-faire policy would result in
an increased number of infections in parallel with the density of VRE. Moreover, although so far unsuccessful, dissemination of VRSA cannot be ruled out should such strains adapt to their new conditions.

Due to its remarkable genome plasticity, further evolution of hospital-adapted *E. faecium* can be predicted. The fact that the transmission of these clones is mainly confined to healthcare settings provides an opportunity for targeted prevention. Guidelines have been produced, and they have been successful in the control of VRE when systematically applied in all healthcare facilities in a given region, or in countries with low VRE prevalence where control is easier.

Conclusions

Bacteria are not equally equipped when it comes to facing the constraints of the hospital environment. In particular, *E. faecium* is characterized by its remarkable survival abilities in hostile conditions and has used both its inherent genetic patrimony and its genome plasticity to generate subpopulations highly adapted to the specific hospital ecosystem. In marked contrast to other hospital-adapted organisms that are much more pathogenic, this microorganism has developed a colonization strategy. Initially considered harmless, this bacterium has become a major noscomial pathogen, although the emergence of clones highly adapted to hospital settings is probably not the ultimate step of evolution in *E. faecium*.

Much as for Mary Shelley’s Victor Frankenstein, the question is will the MDR monsters that we have created in the hospitals punish us? The answer will depend on our capacity to improve infection control policies and antibiotic usage, to develop new antimicrobials and to conceive new approaches to combat these microorganisms. Modern approaches to the biology and genetics of enterococci will probably allow us to better understand the relationships between *E. faecium* and the host and to find new ways to combat these MDR bacteria. Prevention of gastrointestinal colonization appears to be a key issue in this context.

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References

46 Nallaparedy SR, Weinstock GM, Murray BE. Clinical isolates of Enterococcus faecium exhibit strain-specific collagen binding mediated by Acm, a new member of the MSCRAMM family. Mol Microbiol 2003; 47: 1733–47.


