

Enterococci and Vancomycin Resistance

G. L. French

From the Department of Microbiology, Division of Infection, United Medical and Dental Schools, Guy's and St. Thomas's Hospitals, London, United Kingdom

The frequency of infections with multiply antibiotic-resistant gram-positive bacteria is increasing, and in some cases these organisms remain susceptible only to the glycopeptides vancomycin and teicoplanin. The appearance of transferable high-level glycopeptide resistance in enterococci—producing some strains that are now resistant to all available antibiotics—is thus a cause for concern. The enterococci readily colonize the bowel, spread rapidly among hospital patients, and transfer their antibiotic resistances widely among themselves and other gram-positive species. Glycopeptide resistance has not yet transferred *in vivo* to other significant pathogens, but experimental transfer to *Staphylococcus aureus* has been achieved *in vitro*. The emergence of glycopeptide-resistant enterococci has been encouraged by the increasing use of aminoglycosides, cephalosporins, and quinolones for the treatment of infections due to gram-negative bacteria and glycopeptides for infections due to staphylococci and *Clostridium difficile*. In Europe this antibiotic pressure has been aggravated by the use of the glycopeptide avoparcin in animal feeds. The enterococci may now be poised to disseminate glycopeptide resistance among other more pathogenic gram-positive bacteria.

The Action of Glycopeptides

The glycopeptides inhibit synthesis of gram-positive cell walls by binding to the amide bond of the D-alanyl-D-alanine terminal sequences of the muramyl pentapeptide of the elongating peptidoglycan polymer. The large, rigid glycopeptide molecules impede the action of both the polymerase that extends the peptidoglycan backbone and the transpeptidase that cross-links the growing chain to the existing cell wall [1–4]. The glycopeptides thus bind with high specificity to essential structural molecules outside the cell membrane and block several stages in the formation of gram-positive cell walls. These multiple modes of action contribute to the rarity of acquired resistance to vancomycin—a drug that has been in clinical use since 1958—and led to predictions that such resistance was unlikely to occur on any significant scale [3].

Gram-negative bacteria are resistant to glycopeptides, presumably because the large molecules cannot penetrate their outer membranes. Most clinically important gram-positive organisms are susceptible to these drugs, but species of *Mycobacterium*, *Erysipelothrix*, *Leuconostoc*, *Pediococcus*, and *Lactobacillus* and the glycopeptide-producing actinomycetes are usually resistant [5–7]. Vancomycin MICs are usually in the range of 0.5–4.0 mg/L for susceptible bacteria, and teicoplanin MICs are similar or slightly lower [8–10].

Mechanisms of Vancomycin Resistance

Vancomycin resistance can be divided into low-level (MIC, 8–32 mg/L) and high-level (MIC, \geq 64 mg/L), and there are

several resistance phenotypes. The most common phenotypes include (1) low-level teicoplanin resistance in coagulase-negative staphylococci, which usually remain vancomycin-susceptible; (2) inducible low-level vancomycin resistance in enterococci, which usually remain teicoplanin susceptible; and (3) inducible high-level transferable resistance to both vancomycin and teicoplanin, seen in enterococci. Some of the enterococcal phenotypes have been investigated in detail [11, 12].

High-level, inducible, transferable resistance is now called VanA, and inducible low-level vancomycin resistance, VanB. VanA resistance is usually plasmid borne but is now known to be encoded on a transposon that may pass to the chromosome. VanB resistance is usually chromosomal and is occasionally transferable from chromosome to chromosome on a transposon [13–15]. Both VanA and VanB resistance are seen most commonly in *Enterococcus faecium* and *Enterococcus faecalis*.

The constitutive low-level vancomycin resistance seen in some strains of *Enterococcus gallinarum* is called VanC [16]; the similar low-level resistances in *Enterococcus casseliflavus* and *Enterococcus flavescens* are distinct from VanC but have not been fully elucidated. A VanD phenotype has been described in a single strain of *E. faecium* [17]. This organism had constitutive resistance to vancomycin (MIC, 64 mg/L) and to low levels of teicoplanin (MIC, 4 mg/L).

Most outbreaks of vancomycin-resistant enterococci (VRE) have involved the VanA phenotype (inducible high-level transferable resistance to both vancomycin and teicoplanin). In these cases the resistance plasmid often appears in both *E. faecium* and *E. faecalis* and readily transfers to multiple different strains of these species during the outbreak. Several outbreaks of organisms with the VanB phenotype have also been described, more often with single strains. Species exhibiting the VanC phenotype appear sporadically in clinical specimens, but cross-infection with this phenotype is uncommon.

Reprints or correspondence: Dr. G. L. French, Department of Microbiology, Division of Infection, United Medical and Dental Schools, Guy's and St. Thomas's Hospitals, London, SE1 7EH, United Kingdom.

Clinical Infectious Diseases 1998;27(Suppl 1):S75–83

© 1998 by the Infectious Diseases Society of America. All rights reserved.
1058-4838/98/2702-0011\$03.00

The VanA resistance phenotype has been the most thoroughly studied [11, 12]. Vancomycin MICs range from 64 mg/L to >1,024 mg/L, and teicoplanin MICs are usually one- or twofold lower. VanA resistance has been seen so far only in clinical isolates of enterococci, most frequently in *E. faecium*, sometimes in *E. faecalis*, and rarely in *Enterococcus avium*, *Enterococcus durans*, and some other enterococcal species [11, 12, 18–22]. VanA resistance is usually inducible by subinhibitory concentrations of both vancomycin and teicoplanin and is associated with the production of a 38–40-kD membrane protein called VanA [23, 24] and encoded by the *vanA* gene [25, 26].

The deduced amino acid sequence of VanA indicates that it is related to the bacterial D-Ala:D-Ala ligases, which mediate the D-alanyl-D-alanine (D-Ala-D-Ala) linkages of the growing cell wall peptidoglycan [26]. VanA appears to be a D-Ala:D-X ligase of relaxed substrate specificity that can condense D-alanine with other amino acids, fatty acids, or hydroxy acids [27–30]. In resistant organisms, D-Ala-D-Ala is replaced with D-Ala-D-lactate (D-Lac), which cannot bind glycopeptides [30–32].

The production of D-Ala-D-Lac depends on the cooperative activity of three enzymes, VanA, VanH, and VanX. VanH is a lactate dehydrogenase that produces excessive lactic acid from pyruvate, favoring the formation of D-Ala-D-Lac bonds by VanA [30, 33]. VanX is a D, D-dipeptidase that destroys any D-Ala-D-Ala bonds produced by the normal bacterial ligase.

In vancomycin-resistant *E. faecalis* and *E. faecium*, the genes encoding these proteins are located on a plasmid and arranged sequentially within an operon. Induction of resistance is mediated by the transcription activator *vanR* and the membrane sensor *vanS*, located upstream of the other members of the gene cluster [34]. The *vanA* operon may have evolved as part of a system of nutritional adaptability. In this model, membrane sensors (NtrB, EnvZ, PhoR, and VanS) detect changes in environmental concentrations (of nitrogen, osmolality, phosphate, and vancomycin/D-alanine) and switch on response regulators (NtrC, OmpR, PhoB, and VanR) to activate operons that enable alternative nutritional pathways [35]. In the case of *vanA*, this would allow cell wall formation in the presence of low external concentrations of D-alanine or high concentrations of vancomycin.

The *vanA* gene cluster also contains the genes *vanY* and *vanZ*, which, in contrast to *vanA*, *vanH*, *vanX*, *vanS*, and *vanR*, are not essential for the expression of vancomycin resistance. *vanY* encodes a D,D-carboxypeptidase that can cleave excess D-Ala-D-Ala bonds not hydrolyzed by VanX; in this way it may act as a fail-safe mechanism to ensure the expression of vancomycin resistance in organisms growing in the presence of high concentrations of D-alanine. The VanZ peptide confers low-level teicoplanin resistance by an unknown mechanism.

The *vanA* gene cluster thus consists of seven genes arranged in the sequence *vanR*, *vanS*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ*. In *E. faecium* BM4147, these genes are contained in a transposon Tn1546, 10,851 bp in length, and preceded by ORF1 (open reading frame) and ORF2, which encode a transposase and a resolvase, respectively [36] (figure 1). The whole

transposon is flanked by inverted repeats IR_L and IR_R. Although usually encoded on a plasmid, the transposon has, in some cases, transferred to the chromosome [37]. A recently discovered transposon, Tn5482, consisting of Tn1546 and insertion sequences IS1251, can transfer *vanA* from the chromosome of one enterococcus to another [38].

The mechanisms and genetics of the other vancomycin resistance phenotypes have not been so well elucidated, but they all seem to result from the production of altered ligases. The VanB phenotype is encoded by a similar gene cluster [39, 40] encoded on a transposon Tn1547 and containing the *vanB* gene. The gene product VanB encodes a ligase that has a 76% amino acid identity with VanA [39] and is presumably responsible for the formation of D-Ala-D-Lac [41, 42]. Enterococci expressing VanB are resistant to vancomycin but remain susceptible to teicoplanin, presumably because teicoplanin does not induce resistance [40]. *vanB* can self-transfer between enterococci by conjugation, with the genetic material usually moving directly between the chromosomes [14, 38]. *vanC* in *E. gallinarum* encodes a ligase that substitutes D-Ala-D-Serine for the normal D-Ala-D-Ala in peptidoglycan precursors [43]. The *vanD* gene encodes a D-Ala:D-Lac ligase related to VanA and VanB [17].

Where Has *vanA* Come From?

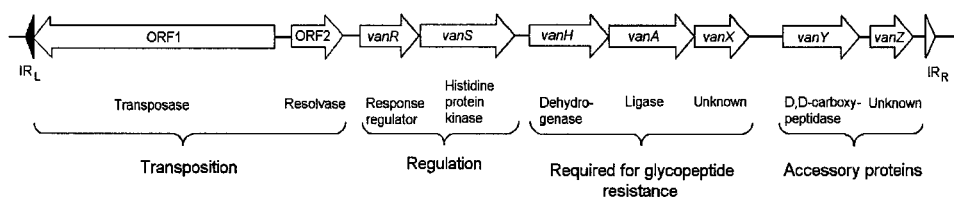
The emergence of the *vanA* cluster, with its cooperative genes arranged in perfect functional sequence, is surprising, and attempts have been made to determine where this transposon might have come from. However, further analysis only deepens the mystery. Genetic probes for *vanA* hybridize with DNA from enterococcal strains with the VanA phenotype but not with strains showing low-level (VanB) resistance, nor with *E. gallinarum* that shows the VanC phenotype or with DNA from the organisms that produce vancomycin and teicoplanin, *Amycolatopsis orientalis* and *Actinoplanes teichomyceticus* [27]. VanB and VanC also appear to be ligases, but they have only a 76% and 38% amino acid homology, respectively, with VanA. The naturally vancomycin-resistant species *Lactobacillus*, *Pediococcus*, and *Leuconostoc* also appear to produce peptidoglycan precursors that terminate in D-Ala-D-Lac [41, 44], but the ligases of these organisms have only ~30% amino acid homology with VanA and VanB [45].

The guanine and cytosine (G+C) content of *E. faecalis* and *E. faecium* are 38% and 39%, respectively, while the essential genes of the *vanA* cluster have G+C contents of 41%–45%, and the *vanB* gene has a G+C content of 49%. The associated genes *vanY* and *vanZ* and the ORF genes of the transposase and resolvase have G+C contents ranging from 29% to 37%. The accumulated evidence, therefore, does not indicate an obvious origin for the *vanA* gene cluster, and the transposon may be from yet another genetically unrelated source.

Transfer of *vanA*

VanA resistance is variably transferable in vitro by conjugation or transformation to *E. faecalis*, *Streptococcus sanguis*,

Figure 1. Map of transposon Tn1546. Adapted from [36]. IR = inverted repeat; ORF = open reading frame.



Streptococcus (Lactococcus) lactis, *Streptococcus pyogenes*, *Listeria monocytogenes*, and, most recently, *Staphylococcus aureus* [18–20, 23, 46, 47]. However, although *vanA* has appeared naturally in enterococci and some poorly pathogenic coryneform bacteria [48], transfer to other bacterial species has not yet occurred in vivo. Transferable vancomycin resistance in enterococci is usually, but not always [21], associated with plasmids of ~30 MDa. Plasmids from different isolates differ in size, phenotypic expression, and restriction digest patterns, but they share genetic homology [27, 49].

Enterococci Are Increasingly Common Causes of Hospital-Acquired Infection

Enterococci colonize the bowels of >90% of healthy humans, and are found in counts of up to 10^7 cfu/g of stool [50, 51]. *E. faecalis* is much more common than *E. faecium*, and the other 16 species of enterococci are found only rarely. *E. faecalis* accounts for ~90% of enterococcal isolates in clinical specimens, but in recent years *E. faecium* has become more common, probably because of its greater antibiotic resistance [52, 53]. Most clinical isolates of enterococci represent colonization rather than infection. They are often found in association with other more virulent organisms, and the most common site of isolation is the urinary tract. However, enterococci can cause more-invasive infection and are sometimes responsible for cholecystitis, cholangitis, peritonitis, septicemia, endocarditis, and meningitis, as well as simple wound infections [52, 53].

For the last two decades, enterococci have been the third most common cause of hospital-acquired infections (HAI) after *Escherichia coli* and *S. aureus* and ahead of *Pseudomonas aeruginosa* [54, 55]. Enterococci are becoming more common and are responsible for 10%–12% of all HAIs, 10%–20% of hospital-acquired urinary tract infections, and 5%–10% of hospital-acquired bacteremias [55, 56].

The reservoir for enterococci is the bowel, and most infections are endogenous. Thus, the increasing isolation rate for enterococci is usually caused by multiple endogenous strains rather than outbreaks of cross-infection. Nevertheless, epidemic infection does occur, and organisms are probably spread from patient to patient on the hands of hospital staff [57–60].

During outbreaks of both vancomycin-susceptible (VSE) and VRE, there is extensive colonization of the bowels of patients and staff, and asymptomatic carriage may persist for months [61–64]. Colonization of other mucous membranes such as the

throat, stomach, and vagina may occur, and skin colonization of moist sites such as the groin has been reported [65]. Organisms may then be transferred from these sites by hand contact, as happens with gram-negative nosocomial pathogens such as *Klebsiella* species [66].

After experimental inoculation, VSE and VRE survive on fingers for ~30 minutes. Washing with soap and water fails to remove these organisms. Aqueous chlorhexidine and povidone iodine are also unreliable agents, but alcohol and alcoholic chlorhexidine are effective [67, 68]. Hospital staff are notoriously poor at hand washing and shun alcoholic preparations that cause chapped skin. It is likely, therefore, that hand transfer is a major route of enterococcal cross-infection. Evidence for this is provided by the isolation of outbreak strains of VRE and VSE from environmental surfaces with likely hand contact, including telephones, stethoscopes, bedrails, countertops, thermometer handles, bedpans, blood pressure cuffs, pulse-oximeter couplings, instrument dials, and door knobs [62, 64, 69–73]. Boyce et al. [64] found that during an outbreak of enterococci carrying transferable VanB resistance, there was extensive contamination of the environment, which was significantly more widespread around colonized patients who also had diarrhea.

Enterococci may survive for ≤ 7 days in the environment [72] and may then be a further source of cross-infection. The recovery of environmental isolates depends on culture methods, and the results must be interpreted with care. Nevertheless, some studies [38, 63, 65, 74] have failed to find epidemic strains of enterococci in the hospital environment, and in many outbreaks these organisms may not be a major source of cross-infection. The detection of environmental contamination may be related to the availability of single rooms for isolation, nursing and domestic cleaning practices, whether or not a patient has diarrhea, the resistance of the outbreak strains to drying, and the methods used for environmental screening.

Enterococci Are Inherently Antibiotic Resistant and Increasingly Multiresistant

Enterococci are inherently more antibiotic resistant than other clinically important gram-positive bacteria and readily acquire additional resistances (table I). They are more resistant to penicillin than the streptococci because of the low affinity of their cell wall penicillin-binding proteins (PBPs) [75]. The MICs of penicillin are 2–8 mg/L for *E. faecalis* and 16–32

Table 1. Antimicrobial resistance mechanisms in enterococci.

Resistance	Characteristic(s)	Genes
Intrinsic		
β -Lactams	Possession of low-affinity PBPs	Chromosomal
Clindamycin	Low-level	Chromosomal
Aminoglycosides	Low-level due to permeability/low uptake	Chromosomal
Trimethoprim	Resistance in vivo due to ability of organism to use exogenous folates	Chromosomal
Quinolones	Permeability/reduced uptake	Chromosomal
Glycopeptides	Low-level in <i>Enterococcus casseliflavus</i> and <i>Enterococcus gallinarum</i>	Chromosomal <i>vanC</i> genes
Acquired		
β -Lactams	Increased resistance due to altered PBPs; high-level resistance due to β -lactamase production	Chromosomal Transposon/plasmid
Aminoglycosides	High-level due to production of AMEs	Transposon/plasmid
Macrolides/lincosamides/streptogramins	Due to enzymes that methylate 23S rRNA, resulting in reduced ribosomal binding	Transposon/plasmid
Tetracycline	Complex, partly due to increased efflux of drug from cell	<i>tet</i> family of genes Transposon/plasmid
Chloramphenicol	Chloramphenicol acetyltransferase	Plasmid
Quinolones	Higher-level resistance due to gyrase mutation	Chromosomal <i>gyrA</i> mutation
Vancomycin	Several phenotypes: high-level resistance due to altered ligase	<i>vanA</i> and <i>vanB</i> genes on transposon/plasmid

NOTE. AMEs = aminoglycoside-modifying enzymes; PBPs = penicillin-binding proteins.

mg/L for *E. faecium*. These organisms are more susceptible to ampicillin, whose MICs are about one dilution lower than those of penicillin. However, ampicillin MBCs are usually much higher than MICs, and clinical therapy may not be bactericidal. For this reason, enterococcal endocarditis is usually treated with the synergistic and bactericidal combination of ampicillin plus gentamicin. However, this therapy will not be effective for the increasingly common strains with high-level aminoglycoside resistance.

Enterococci are usually clinically resistant to cephalosporins, including the newer related β -lactams such as imipenem. These organisms also have inherent low-level resistance to clindamycin, aminoglycosides, and the quinolones, and co-trimoxazole is not bactericidal. The inherent low-level vancomycin resistance in *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* has already been discussed.

In addition to this natural low-level resistance, the enterococci readily acquire high-level resistance and resistance to other drugs. Many recent isolates of *E. faecium* are fully resistant to ampicillin by the production of additional low-affinity PBPs, and some strains of *E. faecalis* produce a plasmid-encoded β -lactamase, similar to the penicillinase of *S. aureus* [76, 77], but such strains are usually uncommon in clinical material. Enterococci are thus increasingly resistant to β -lactams [78]. High-level aminoglycoside resistance may be acquired by chromosomal mutation (altering the ribosomal binding site) or by acquisition of plasmids encoding aminoglycoside-modifying enzymes [79, 80]. Chloramphenicol resistance is also usually enzymatic and plasmid borne, and high-level erythromycin and clindamycin resistance is now commonly seen as a result of the widespread dissemination of transposons encoding macrolide/lincosamide/streptogramin resistance. Tetracycline resistance is also common and is mediated

by a variety of mechanisms, often transferable by plasmids and transposons.

Finally, some strains of enterococci have acquired plasmid-mediated high-level vancomycin resistance as described previously. The gradual accumulation of multiresistance to the whole range of antibiotics normally active against gram-positive bacteria has resulted in some clinical isolates of enterococci—especially *E. faecium*—that are resistant to all currently available agents.

Increasing Use of Antimicrobials Has Encouraged the Emergence of Multiresistant Enterococci and VRE

The increasing frequency with which enterococci are isolated in clinical specimens has corresponded with the increasing use of antibiotics to which these organisms are naturally resistant. In particular, there has been a dramatic increase in the use of cephalosporins and quinolones, largely directed against gram-negative bacteria, which has probably encouraged the emergence of enterococci as nosocomial pathogens. It is difficult to prove this hypothesis, but the circumstantial evidence is convincing. For example, many case-control studies have shown that nosocomial enterococcal infection is associated with prior antimicrobial therapy, especially that with cephalosporins [81, 82], and studies on new agents have repeatedly shown that superinfection with enterococci often occurs following therapy with β -lactams and related drugs (moxalactam, aztreonam, or imipenem) and quinolones (ciprofloxacin) [83–85].

The facts that *E. faecium* is inherently more resistant than *E. faecalis* and that *E. faecium* has greater ability to acquire resistance, especially to ampicillin, are probably responsible for the increasing frequency of this organism as a cause of

HAI. Since ampicillin resistance in this species is usually not due to β -lactamase production, the increasing use of β -lactam/ β -lactamase inhibitor combinations such as amoxicillin/clavulanic acid and ampicillin/sulbactam has probably also contributed to its growing importance.

There must have been an element of chance in the initial emergence of vancomycin resistance, but once the VanA plasmid appeared, its dissemination has been encouraged by increasing usage of glycopeptides. Evidence for this is shown by the fact that VRE most frequently cause outbreaks in special units (i.e., adult and neonatal intensive care units, hematologic and oncologic wards, and renal and liver transplantation units) where glycopeptides are widely used. There has also been a more general increase in glycopeptide use in many other areas. Although vancomycin has been available for many years, the availability of less toxic and cheaper drugs limited the use of this agent until ~ 10 years ago. Since then, the use of glycopeptides for the treatment of infections due to multiresistant gram-positive bacteria has greatly increased, especially those due to staphylococci. Methicillin-resistant *S. aureus* is now widespread throughout the world as a cause of hospital-acquired and, increasingly, community-acquired infection, and vancomycin is the treatment of choice for invasive infection with this organism [86].

Coagulase-negative staphylococci have also become very common because of their ability to stick to plastic catheters and other devices [87]. In hospitals, $\sim 50\%$ of coagulase-negative staphylococci are resistant to methicillin and other antibiotics [88, 89], and this finding has encouraged clinicians to administer vancomycin—sometimes unnecessarily—when catheter-associated sepsis is suspected. Inappropriate glycopeptide use has been further encouraged by the marketing of teicoplanin, which is nontoxic and therefore more readily used than vancomycin. Finally, oral vancomycin is widely used for the eradication of *Clostridium difficile* in suspected cases of pseudomembranous colitis, another nosocomial infectious disease whose frequency has increased markedly in the last decade [90].

These general trends are well illustrated by the study of Ena and colleagues [91] of intravenous vancomycin use in a university hospital over the 10-year period 1981–1991. Vancomycin use increased 20-fold over this period, and the increase was almost linear. Use was primarily related to the presence of indwelling intravascular devices and was particularly common among hematology/oncology patients. About one-third of courses were given for prophylaxis, one-third for empirical therapy, and only one-third for specific therapy directed by culture and susceptibility results.

Glycopeptide Use in Animal Husbandry

While investigating a hospital outbreak of a clone of vancomycin-resistant *E. faecium* in a renal unit in Oxford, United Kingdom, Jordens and colleagues [92] found fecal carriage of a variety of VRE strains in nonrenal patients in the hospital

and in the community. This prompted these authors to investigate the distribution of VRE in nonhuman sources, and they found 14 different ribotypes of VRE in raw sewage, farm animals (including a duck, a chicken, a turkey, a dog, and a pony, as well as a number of pigs), and uncooked shop-bought chickens [93]. All isolates had high-level vancomycin resistance and contained the *vanA* gene. Klare et al. [94, 95] then reported finding similar VRE in sewage and pig and poultry manure from farms in Germany where the glycopeptide avoparcin was used as a feed supplement but not from a poultry farm where avoparcin was not used. They also found VRE in uncooked chickens delivered to their hospital kitchens. All these organisms contained *vanA*, and they were of a variety of different molecular types.

Torres et al. [96] found *vanA*-mediated VRE in sewage in Spain, and, in an extensive series of studies in Denmark, Aarestrup [97] found more VRE in poultry and pig farms where avoparcin feed supplements were used but not in farms where such supplements were not being used. Molecular typing of these Danish isolates showed a wide range of types, but some were similar in both animals and humans. The relationship between avoparcin use in animals and the appearance of vancomycin-resistant enterococci in humans has been reviewed in detail by Bates [98].

The results from these European studies provide incontrovertible evidence that *vanA*-mediated VRE are widespread in the community and flourish in the bowels of animals where avoparcin is used as a feed supplement. It is likely that some human isolates of VRE have originated from animal sources. The use of avoparcin appears to make a significant contribution to the antibiotic pressure on VRE, and Denmark banned its use in animals in May 1995. Avoparcin is not used as an animal feed supplement in the United States, where VRE do not appear to arise in the community.

Outbreaks of VRE

The first report of VRE expressing the VanA phenotype appeared in 1988 when Uttley and colleagues [21, 99] described a large outbreak that affected 41 renal patients at Dulwich Hospital in South London between 1986 and 1988. Although most isolates were *E. faecium*, some were *E. faecalis*, and some *E. avium*. The most common site of isolation was the urinary tract, but there were many deep infections, and eight patients had bacteremia. Around the same time, there were sporadic isolations of similar organisms in Paris and Nancy, France: LeClerq et al. [18, 46] reported phenotypically distinct strains of *E. faecium* from the stools of four leukemic patients; Shlaes and colleagues [19] isolated *E. faecium* from the blood and peritoneal abscess of a patient with peritonitis and *E. faecalis* from the urine of a patient with a recurrent urinary tract infection [20]; in addition, Bingen and colleagues [100] isolated vancomycin-resistant *E. faecium* from the blood of one patient and from the stools of 14 others. The latter outbreak involved

four wards of a pediatric hospital over a 17-month period between 1988 and 1990, but the isolates were all genetically distinct by restriction digest analysis.

VRE appeared in New York City in 1989 [101]. The first isolate was reported in September 1989, and by October 1991, resistant strains had been isolated from >360 patients in at least 38 hospitals. Most isolates were *E. faecium*, and a few were *E. faecalis*. The great majority were hospital acquired, but two of the first 100 patients affected seem to have acquired their infections in the community. Forty-two patients died, and vancomycin-resistant enterococcal infection was believed to have contributed to 19 of these deaths. Among 23 isolates examined by endonuclease restriction, there were 14 distinct strains. Most isolates were of the VanA phenotype, but these showed three different probing patterns, and the authors believed that this extensive and continuing outbreak was the result of dissemination of a transposon.

Since these early reports, increasing numbers of outbreaks of VRE have been reported from North America and Europe [38, 61–65, 70, 74, 82, 92, 102]. The U.S. National Nosocomial Infection Surveillance survey found that there was a 20-fold increase in the number of nosocomial VRE isolates during the period 1989–1993 [103]. VRE were responsible for 0.3% of all nosocomial infections in 1989, and this percentage rose to 7.9% in 1993. VRE accounted for 0.4% of urinary tract infections in 1989 and 13.6% in 1993. By the end of the period, 3.8% of blood isolates of enterococci were vancomycin resistant, and the mortality associated with bacteremias caused by resistant strains was 37% compared with 16% for susceptible ones. A similar trend has been noted in the United Kingdom [104].

From the earliest reports, most outbreaks have shared certain typical features. These outbreaks have tended to occur in special units where compromised patients were receiving care and where there is a high level of cephalosporin and glycopeptide use. Most isolates have represented colonization, but in ~10% of affected patients, serious invasive infection has occurred, often producing bacteremia and associated with a high mortality. In addition to clinical cases, screening has revealed many asymptomatic patients (and sometimes staff) with stool carriage of VRE. The environment surrounding affected patients is sometimes (but not always) extensively contaminated with VRE, as discussed previously. Several distinct VRE are usually circulating, commonly including more than one species and with multiple types within each species. However, in some outbreaks, a single strain predominates [80]. The predominant outbreak organisms are *E. faecium*, which make up ~80%–90% of isolates; the remainder are usually *E. faecium*. The resistance phenotype is nearly always VanA, and there is often evidence of strain-to-strain transfer of plasmids (and transposons) encoding the *vanA* gene.

Where Next for Vancomycin Resistance?

Enterococci appear to have evolved perfectly to spread and transmit vancomycin resistance genes and other resistance

genes within both the hospital and the community. Because they are of low virulence and readily colonize the bowels of humans and animals, asymptomatic carriers are common and can cause extensive contamination of the environment. Although it has not yet occurred in nature, there is a real danger that in the future *vanA* resistance will spread from enterococci to staphylococci and pneumococci. The emergence of vancomycin-resistant, methicillin-resistant *S. aureus* now seems inevitable, and we face the prospect that serious and invasive staphylococcal infections may once more become untreatable. There is thus an urgent need to reduce the antibiotic pressure that encourages such resistance and to limit the spread of the vancomycin-resistant enterococci that disseminate it.

Outbreaks should be dealt with by isolation of patients and hand washing; antibiotic pressure should be reduced by restricting the clinical use of broad-spectrum cephalosporins, quinolones, and glycopeptides; the use of avoparcin in animal feeds should be critically reviewed and probably banned; and methods should be sought to eliminate stool carriage of resistant organisms. The recent Hospital Infection Control Practices Advisory Committee document (1995) [105] has rightly emphasized the importance of education in the battle against vancomycin-resistant organisms. All individuals involved with antibiotic use and infection control, whether they are medical, nursing, scientific, pharmaceutical, agricultural, managerial, or political professionals, should be made aware of the seriousness of this situation and work together to prevent what may otherwise be a medical disaster.

References

1. Barna JC, Williams DH. The structure and mode of action of glycopeptides of the vancomycin group. *Ann Rev Microbiol* **1984**;34:339–57.
2. Nagarajan R. Antibacterial activities and modes of action of vancomycin and related glycopeptides. *Antimicrob Agents Chemother* **1991**;35:605–9.
3. Reynolds PE. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur J Clin Microbiol Infect Dis* **1989**;8:943–50.
4. Reynolds PE. Glycopeptide resistance in Gram-positive bacteria. *J Med Microbiol* **1992**;36:14–7.
5. Orberg PK, Sandine WE. Common occurrence of plasmid DNA and vancomycin resistance of *Leuconostoc* spp. *Appl Environ Microbiol* **1984**;48:1129–33.
6. Colman G, Efstratiou A. Vancomycin-resistant leuconostocs, lactobacilli and now pediococci. *J Hosp Infect* **1987**;10:1–3.
7. Courvalin P. Resistance of enterococci to glycopeptides. *Antimicrob Agents Chemother* **1990**;34:2291–6.
8. Cooper GL, Given DB. Vancomycin. A comprehensive review of 30 years of clinical experience. New York: Park Row Publishers, **1986**.
9. Watanakunakorn C. Mode of action and in-vitro activity of teicoplanin. *J Antimicrob Chemother* **1984**;14:(suppl D)7–18.
10. Wilson APR, Grünenberg RN. Teicoplanin. The first decade. Abingdon, UK: The Medicine Group, **1997**.
11. Woodford N, Johnson AP, Morrison D, Speller DCE. Current perspectives on glycopeptide resistance. *Clin Microbiol Rev* **1995**;8:585–615.
12. Leclercq R, Courvalin P. Resistance to glycopeptides in enterococci. *Clin Infect Dis* **1997**;24:545–56.
13. Quintiliani R Jr, Evers S, Courvalin P. The *vanB* gene confers various levels of self-transferable resistance to vancomycin in enterococci. *J Infect Dis* **1993**;167:1220–3.

14. Quintillian R, Courvalin P. Conjugal transfer of the vancomycin resistance determinant *vanB* between enterococci involves the movement of large genetic elements from chromosome to chromosome. *FEMS Microbiol Lett* **1994**;119:359–64.
15. Quintillian R, 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**;172:1–8.
16. Leclerq R, Dutka-Malen S, Duval J, Courvalin P. Vancomycin resistance gene *vanC* is specific to *Enterococcus gallinarum*. *Antimicrob Agents Chemother* **1992**;36:2005–8.
17. Perichon B, Reynolds P, Courvalin P. VanD-type glycopeptide-resistant *Enterococcus faecium* BM4339. *Antimicrob Agents Chemother* **1997**;41:2016–8.
18. Leclerq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Engl J Med* **1988**;319:157–61.
19. Shlaes DM, Al-Obeid S, Shlaes JH, Boisivon A, Williamson R. Inducible, transferable resistance to vancomycin in *Enterococcus faecium* D399. *J Antimicrob Chemother* **1989**;23:503–8.
20. Shlaes DM, Bouvet A, Devine C, Shlaes JH, Al-Obeid S, Williamson R. Inducible, transferable resistance to vancomycin in *Enterococcus faecalis* A256. *J Antimicrob Chemother* **1989**;23:503–8.
21. Uttley AHC, George RC, Naidoo J, et al. High-level vancomycin-resistant enterococci causing hospital infections. *Epidemiol Infect* **1989**;103:173–81.
22. Gopal Rao G, Morris KJ, George RC, Woodford N. Vancomycin-resistant enterococci in a district general hospital. *Lancet* **1992**;340:1471–2.
23. Nicas TI, Wu CYE, Hobbs JR, Preston DA, Allen NE. Characterization of vancomycin resistance in *Enterococcus faecium* and *Enterococcus faecalis*. *Antimicrob Agents Chemother* **1989**;33:1121–4.
24. Williamson R, Al-Obeid S, Shlaes JH, Goldstein FW. Inducible resistance to vancomycin in *Enterococcus faecium* D366. *J Infect Dis* **1989**;159:1095–104.
25. Brisson-Noël, Dutka-Malen S, Molinas C, Leclerq R, Courvalin P. Cloning and heterospecific expression of the resistance determinant *vanA* encoding high-level resistance to glycopeptides in *Enterococcus faecium* BM4147. *Antimicrob Agents Chemother* **1990**;34:924–7.
26. Dutka-Malen S, Molinas C, Arthur M, Courvalin P. The VANA glycopeptide resistance protein is related to D-alanyl-D-alanine ligase cell wall biosynthesis enzymes. *Mol Gen Genet* **1990**;224:264–72.
27. Dutka-Malen S, Leclerq R, Courant V, Duval J, Courvalin P. Phenotypic and genotypic heterogeneity of glycopeptide resistance determinants in Gram-positive bacteria. *Antimicrob Agents Chemother* **1990**;34:1875–9.
28. Bugg TDH, Dutka-Malen S, Arthur M, Courvalin P, Walsh T. Identification of vancomycin resistance protein VanA as D-alanine:D-alanine ligase of altered substrate specificity. *Biochemistry* **1991**;30:2017–21.
29. Bugg TDH, Wright G, Dutka-Malen S, Arthur M, Courvalin P, Walsh T. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin-resistance proteins VanH and VanA. *Biochemistry* **1991**;30:10408–15.
30. Arthur M, Molinas C, Bugg TDH, Wright GD, Walsh CT, Courvalin P. Evidence for in vivo incorporation of D-lactate into peptidoglycan precursors of vancomycin-resistant enterococci. *Antimicrob Agents Chemother* **1992**;36:867–9.
31. Messer J, Reynolds PE. Modified peptidoglycan precursors produced by glycopeptide-resistant enterococci. *FEMS Microbiol Lett* **1992**;94:195–200.
32. Allen NE, Hobbs JN, Richardson JM, Riggan RM. Biosynthesis of modified peptidoglycan precursors by vancomycin resistant *Enterococcus faecium*. *FEMS Microbiol Lett* **1992**;98:109–16.
33. Arthur M, Molinas C, Dutka-Malen S, Courvalin P. Structural relationship between the vancomycin resistance protein VanH and 2-hydroxy-carboxylic acid dehydrogenases. *Gene* **1991**;103:133–4.
34. Arthur M, Molinas C, Courvalin P. The VanS-VanR two-component regulatory system controls synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* **1992**;174:2582–91.
35. Parkinson JS, Kofoid EC. Communication modules in bacterial signalling proteins. *Ann Rev Genet* **1992**;26:71–112.
36. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterisation of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* **1993**;175:117–27.
37. Woodford N, Morrison D, Johnson AP, Briant V, George RC, Cookson B. Application of DNA probes for rRNA and *vanA* genes to investigation of a nosocomial cluster of vancomycin-resistant enterococci. *J Clin Microbiol* **1993**;31:653–8.
38. Handwerger S, Skoble J. Identification of chromosomal mobile element conferring high-level vancomycin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* **1995**;39:2446–53.
39. Evers S, Reynolds PE, Courvalin P. Sequence of the *vanB* and *ddl* genes encoding D-alanine:D-lactate and D-alanine:D-alanine ligases in vancomycin-resistant *Enterococcus faecalis* V583. *Gene* **1994**;140:97–102.
40. Evers S, Courvalin P. Regulation of VanB-type vancomycin resistance gene expression by the VanS(B)-VanR(B) two-component regulatory system in *Enterococcus faecalis* V583. *J Bacteriol* **1996**;178:1302–9.
41. Billot-Klein D, Gutmann L, Sable S, Guittet E, van Heijenoort J. Modification of peptidoglycan precursors is a common feature of low-level vancomycin-resistant VANB-type *Enterococcus* D366 and of the naturally glycopeptide-resistant species *Lactobacillus casei*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, and *Enterococcus gallinarum*. *J Bacteriol* **1994**;176:2398–405.
42. Billot-Klein D, Shlaes D, Bryant D, Bell D, van Heijenoort J, Gutmann L. Peptidoglycan structure of *Enterococcus faecium* expressing vancomycin resistance of the VanB type. *Biochem J* **1996**;313:711–5.
43. Reynolds PE, Snaith HA, Maguire AJ, Dutka-Malen S, Courvalin P. Analysis of peptidoglycan precursors in vancomycin-resistant *Enterococcus gallinarum* BM4147. *Biochem J* **1994**;301:5–8.
44. Handwerger S, Pucci MJ, Volk KJ, Liu J, Lee MS. Vancomycin-resistant *Leuconostoc mesenteroides* and *Lactobacillus casei* synthesize cytoplasmic peptidoglycan precursors that terminate in lactate. *J Bacteriol* **1994**;176:260–4.
45. Elisha BG, Courvalin P. Analysis of genes encoding D-alanine:D-alanine ligase-related enzymes in *Leuconostoc mesenteroides* and *Lactobacillus* spp. *Gene* **1995**;152:79–83.
46. Leclerq R, Derlot E, Weber M, Duval J, Courvalin P. Transferable vancomycin and teicoplanin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* **1989**;33:10–5.
47. Noble WC, Virani Z, Cree RGA. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett* **1992**;93:195–8.
48. Power EGM, Abdulla YH, Talsania HG, Spice W, Aathithan S, French GL. *vanA* genes in vancomycin-resistant clinical isolates of *Oerskovia turbata* and *Arcanobacterium (Corynebacterium) haemolyticum*. *J Antimicrob Chemother* **1995**;36:595–606.
49. Handwerger S, Pucci MJ, Kolokathis A. Vancomycin resistance is encoded on a pheromone response plasmid in *Enterococcus faecium* 228. *Antimicrob Agents Chemother* **1990**;34:358–60.
50. Mead GC. Streptococci in the intestinal flora of man and other non-ruminant animals. In: Skinner FA, Quesnel LB, eds. *Streptococci*. London: Academic Press, **1978**:245–61.
51. Noble CJ. Carriage of Group D streptococci in the human bowel. *J Clin Pathol* **1978**;31:1182–6.
52. Murray BE. The life and times of the *Enterococcus*. *Clin Microbiol Rev* **1990**;3:46–65.
53. Moellering RC Jr. Emergence of enterococcus as a significant pathogen. *Clin Infect Dis* **1992**;14:1173–8.
54. Centers for Disease Control. Nosocomial infection surveillance 1984. *MMWR Morb Mortal Wkly Rep* **1986**;35(SS 1):17SS–29SS.

55. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infections. *Am J Med* **1991**;91:(72S–5S).
56. Chenoweth CE, Schaberg DR. Enterococcus species. In: Mayhall GG, ed. Hospital epidemiology and infection control. Baltimore: Williams & Wilkins, **1996**:334–45.
57. Coudron PE, Mayhall CG, Facklam RR, et al. *Streptococcus faecium* outbreak in a neonatal intensive care unit. *J Clin Microbiol* **1984**;20:1044–8.
58. Zervos MJ, Kauffman CA, Therasse PM, Bergman AG, Mikesell T, Schaberg DR. Nosocomial infection by gentamicin-resistant *Streptococcus faecalis*—an epidemiologic study. *Ann Intern Med* **1987**;106:687–91.
59. Zervos MJ, Terpenning MS, Schaberg DR, Therasse PM, Medendorp SV, Kauffman CA. High-level aminoglycoside-resistant enterococci—colonization of nursing home and acute care hospital patients. *Arch Intern Med* **1987**;147:1591–4.
60. Rhinehart E, Smith NE, Wennersten C, et al. Rapid dissemination of β -lactamase-producing, aminoglycoside-resistant *Enterococcus faecalis* among patients and staff in an infant-toddler surgical ward. *N Engl J Med* **1990**;323:1814–8.
61. Guiot HF, Peetermans WE, Sebens FW. Isolation of vancomycin-resistant enterococci in haematologic patients. *Eur J Clin Microbiol Infect Dis* **1991**;10:32–4.
62. Livornese LL, Dias S, Samuel C, et al. Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic thermometers. *Ann Intern Med* **1992**;117:112–6.
63. Rubin LG, Tucci V, Cerenado E, Eliopoulos G, Isenberg HD. Vancomycin-resistant *Enterococcus faecium* in hospitalized children. *Infect Control Hosp Epidemiol* **1992**;13:700–5.
64. Boyce JM, Opal SM, Chow JW, et al. Outbreak of multidrug-resistant *Enterococcus faecium* with transferable *vanB* class vancomycin resistance. *J Clin Microbiol* **1994**;32:1148–53.
65. Handwerker S, Raucher B, Altarac D, et al. Nosocomial outbreak due to *Enterococcus faecium* highly resistant to vancomycin, penicillin, and gentamicin. *Clin Infect Dis* **1993**;16:750–5.
66. Casewell MW, Phillips I. Hands as route of transmission for *Klebsiella* species. *Br Med J* **1977**;2:1315–7.
67. Wade JJ, Desai N, Casewell MW. Hygienic hand disinfection for the removal of epidemic vancomycin-resistant *Enterococcus faecium* and gentamicin-resistant *Enterobacter cloacae*. *J Hosp Infect* **1991**;18:211–8.
68. Kjolen H, Andersen BM. Handwashing and disinfection of heavily contaminated hands—effective or ineffective? *J Hosp Infect* **1992**;21:61–71.
69. Chirurgi VA, Oster SE, Goldberg AA, McCabe RE. Nosocomial acquisition of beta-lactamase–negative, ampicillin-resistant enterococcus. *Arch Intern Med* **1992**;152:1457–61.
70. Karanfil LV, Murphy M, Josephson A, et al. A cluster of vancomycin-resistant *Enterococcus faecium* in an intensive care unit. *Infect Control Hosp Epidemiol* **1992**;13:195–200.
71. Chadwick PR, Oppenheim BA. Vancomycin-resistant enterococci and bedpan washer machines [letter]. *Lancet* **1994**;344:685.
72. Stosor V, Frauchiger W, Cooper I, Peterson L, Noskin G. Survival of enterococci on environmental surfaces [abstract L-42]. In: Abstracts of the 94th General Meeting of the American Society for Microbiology (ASM). Washington, DC: American Society for Microbiology, **1994**.
73. Yamaguchi E, Valena F, Smith SM, Simmons A, Eng RHK. Colonization pattern of vancomycin-resistant *Enterococcus faecium*. *Am J Infect Control* **1994**;22:202–6.
74. Chenoweth CE, Bradley SF, Terpenning MS, et al. Colonization and transmission of high-level gentamicin-resistant enterococci in a long-term care facility. *Infect Control Hosp Epidemiol* **1994**;15:703–9.
75. Williamson R, LeBouguenec C, Guttman L, et al. One or two low affinity penicillin-binding proteins may be responsible for the range of susceptibility of *Enterococcus faecium* to benzylpenicillin. *J Gen Microbiol* **1985**;131:1933.
76. Murray BE. β -lactamase-producing Enterococci. *Antimicrob Agents Chemother* **1992**;37:2355–9.
77. Wells VD, Wong ES, Murray BE, Coudron PE, Williams DS, Markowitz SM. Infections due to beta-lactamase-producing high-level gentamicin-resistant *Enterococcus faecalis*. *Ann Intern Med* **1992**;116:285–9.
78. Grayson ML, Eliopoulos GM, Wennerstein CB, et al. Increasing resistance to β -lactam antibiotics among clinical isolates of *Enterococcus faecium*: a 22-year review at one institution. *Antimicrob Agents Chemother* **1991**;35:2180–4.
79. Krogstad DJ, Korfhagen TR, Moellering RC, Wennerstein C, Swartz MN. Aminoglycoside-inactivating enzymes in clinical isolates of *Streptococcus faecalis*: an explanation for bacterial synergism. *J Clin Invest* **1978**;62:480–6.
80. Eliopoulos GM, Farber BF, Murray BE, Wennersten C, Moellering RC. Ribosomal resistance of clinical enterococcal isolates to streptomycin. *Antimicrob Agents Chemother* **1984**;32:1528–32.
81. Morrison AJ, Wenzel RP. Nosocomial urinary tract infections due to enterococcus: ten years' experience at a university hospital. *Arch Intern Med* **1986**;146:1549–51.
82. Edmond MB, Ober JF, Weinbaum DL, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. *Clin Infect Dis* **1995**;20:1126–33.
83. Moellering RC Jr. Enterococcal infections in patients treated with moxalactam. *Rev Infect Dis* **1982**;4(suppl):S708–11.
84. Chandrasekar PH, Smith BR, LeFrock JL, Carr B. Enterococcal superinfection and colonization with aztreonam therapy. *Antimicrob Agents Chemother* **1984**;26:280–2.
85. Zervos MJ, Bacon AE, Patterson JE, Schberg DR, Kauffman CA. Enterococcal superinfection in patients treated with ciprofloxacin. *J Antimicrob Chemother* **1988**;21:113–5.
86. French GL, Cheng AFB, Ling JML, Mo P, Donnan S. Hong Kong strains of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* have similar virulence. *J Hosp Infect* **1990**;15:117–25.
87. Hamory BH, Parisi JT, Hutton JP. *Staphylococcus epidermidis*: a significant nosocomial pathogen. *Am J Infect Control* **1987**;15:59–74.
88. Christensen GD, Bisno AL, Parisi JT, McLaughlin B, Hester MG, Luther RW. Nosocomial septicemia due to multiply antibiotic-resistant *Staphylococcus epidermidis*. *Ann Intern Med* **1982**;96:1–10.
89. Refsahl K, Andersen BM. Clinically significant coagulase-negative staphylococci: identification and resistance patterns. *J Hosp Infect* **1992**;22:19–31.
90. Report. *Clostridium difficile* infection. Prevention and management. Department of Health and Public Health Laboratory Service (UK). **1994**.
91. Ena J, Dick RW, Jones RN, Wenzel RP. The epidemiology of intravenous vancomycin usage in a university hospital. A 10-year study. *JAMA* **1993**;269:598–602.
92. Jordens JZ, Bates J, Griffiths DT. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. *J Antimicrob Chemother* **1994**;34:515–28.
93. Bates J, Jordens JZ, Griffiths DT. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infections in man. *J Antimicrob Chemother* **1994**;34:507–16.
94. Klare I, Heier H, Claus H, Witte W. Environmental strains of *Enterococcus faecium* with inducible high-level resistance to glycopeptides. *FEMS Microbiol Lett* **1993**;80:23–9.
95. Klare I, Heier H, Claus H, Reissbrodt R, Witte W. *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. *FEMS Microbiol Lett* **1995**;125:165–72.
96. Torres C, Reguera JA, Sanmartin MJ, Pérez-Díaz JC, Baquero F. *vanA*-mediated vancomycin-resistant *Enterococcus* spp. in sewage. *J Antimicrob Chemother* **1994**;33:553–61.
97. Aarestrup FM. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. *Microb Drug Res* **1995**;1:255–7.

98. Bates J. Epidemiology of vancomycin-resistant enterococci in the community and the relevance of farm animals to human infection. *J Hosp Infect* **1997**;37:89–101.
99. Uttley AHC, Collins CH, Naidoo J, George RC. Vancomycin-resistant enterococci [letter]. *Lancet* **1988**;1:57–8.
100. Bingen EH, Denamur E, Lambert-Zechovsky NY, Elion L. Evidence for the genetic unrelatedness of nosocomial vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. *J Clin Microbiol* **1991**;29:1888–92.
101. Frieden TR, Munsiff SS, Low D, et al. Emergence of vancomycin-resistant enterococci in New York City. *Lancet* **1993**;342:76–9.
102. Issack MI, Power EGM, French GL. Investigation of an outbreak of vancomycin-resistant *Enterococcus faecium* by random amplified polymorphic DNA (RAPD) assay. *J Hosp Infect* **1996**;33:191–200.
103. Centers for Disease Control and Prevention. Nosocomial enterococci resistant to vancomycin—United States, 1989–1993. *MMWR Morb Mortal Wkly Rep* **1993**;42:597–9.
104. Anonymous. Vancomycin resistant enterococci in hospitals in the United Kingdom. *Comm Dis Rep CDR Wkly* **1995**;50:281.
105. HICPAC (The Hospital Infection Control Practices Advisory Committee). Recommendations for preventing the spread of vancomycin resistance. *Am J Infect Control* **1995**;23:87–94.