Vancomycin-resistant enterococci: consequences for therapy and infection control

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ABSTRACT

Vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens, initially in the USA, but now also in Europe, where hospital outbreaks are being reported with increasing frequency, although the incidence of VRE infections remains extremely low in most European countries. The recently demonstrated in-human transmission of vancomycin resistance from VRE to methicillin-resistant *Staphylococcus aureus* (MRSA) in two American patients underscores the potential danger of a coexisting reservoir of both pathogens. As MRSA is already endemic in many European hospital settings, prevention of endemicity with VRE seems relevant, but should be balanced against the costs associated with the implementation of effective strategies. The presence of a large community reservoir of VRE in Europe could hamper the feasibility of infection control strategies. Although the prevalence of colonisation amongst healthy subjects has apparently decreased after the ban on avoparcin use in the agricultural industry, a large proportion of admitted patients are still potential sources of VRE transmission. With no risk profile available to identify these carriers, effective screening, followed by barrier precautions for carriers, seems to be impossible. Recent studies, however, have suggested that hospital outbreaks are almost exclusively caused by specific genogroups of VRE that can be characterised phenotypically and genotypically (e.g., co-resistance to ampicillin and the presence of the variant *esp* gene). Based on our own experience, we propose that VRE infection control programmes should be restricted to patients colonised with these VRE strains. If such a strain is cultured from a clinical sample, surveillance amongst contact patients is recommended and barrier precautions should be implemented in the case of documented spread.

Keywords Antibiotic resistance, epidemiology, infection control, review

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INTRODUCTION

Enterococci are the most common aerobic Gram-positive cocci in the bowel and lower female genital tract flora of humans and animals. Initially thought of as merely harmless commensal microorganisms, enterococci have emerged as significant human pathogens, currently being the third most common nosocomial bloodstream pathogen in the USA [1]. Enterococcal infections occur predominantly in patients with immunodeficiencies, either due to their underlying illness or to immunosuppressive therapy, and in patients with breaches in normal defensive barriers, such as intravascular lines and urinary catheters. It has been reported that 60% of enterococcal infections are nosocomial, with half of them occurring in intensive care units (ICUs), most probably because of the selection of these organisms by the use of broad-spectrum antibiotics, such as cephalosporins, which lack enterococcal activity [2,3].

Although *Enterococcus faecalis* has been reported to be responsible for 80–90% of enterococcal infections and *Enterococcus faecium* for 10–20% [2–4], more recent studies have suggested that the proportion of infections caused by *E. faecium* has

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increased [5]. Unfortunately, little is known about the pathogenic mechanisms or virulence factors of these microorganisms, or how the innate immune system of the host recognises *E. faecium*. Nevertheless, the increasing relevance of enterococci as nosocomial pathogens is at least partly explained by their intrinsic resistance to antibiotic classes (such as cephalosporins, anti-staphylococcal penicillins, clindamycin and trimethoprim) and their natural ability to acquire and exchange genetic elements encoding antibiotic resistance. A striking example of the latter is the development of plasmid-mediated vancomcyin resistance.

The first patients infected with plasmid-mediated vancomycin-resistant enterococci (VRE) were reported from France and England in 1986 [6,7]. The most common phenotype of resistance (*vanA*) is associated with acquired, inducible, high-level resistance to both vancomycin (MIC > 32 mg/L) and teicoplanin (MIC > 16 mg/L), and is carried on a transposon (Tn*1546*) that is transferable to other susceptible enterococci by conjugation. Several acquired glycopeptide-resistant phenotypes have been characterised since then, including *vanB* and the less common *vanD*, *vanE* and *vanG* types [8]. The *vanB* phenotype, which is chromosomally mediated, inducible and transferable by conjugation, mediates inducible resistance to vancomycin, but not to teicoplanin. However, the development of teicoplanin resistance occurs rapidly during antibiotic exposure [9]. Enterococci harbouring *vanC* genes, such as *E. flavescens* and *E. gallinarum*, are intrinsically resistant to low levels of vancomycin (MIC values of 8–16 mg/L). Remarkably, vancomycin resistance is more common in *E. faecium* than in *E. faecalis* [5,10,11].

Since the initial descriptions, VRE (predominantly the *vanA* phenotype) have emerged as important nosocomial pathogens worldwide [1,12–23]. The first VRE outbreaks in US hospitals often resulted from dissemination of a single strain, especially in ICUs and nephrology wards [24–26]. Subsequently, inter-hospital spread of VRE was observed [27,28], and the monoclonal nature of outbreaks changed to outbreaks characterised by multiple enterococcal strains, finally leading to situations of polyclonal endemicity [13,28–32].

For obvious reasons, the need to control this nationwide nosocomial epidemic of VRE was widely recognised. In 1996, the Hospital Infection Control Practices Advisory Committee (HICPAC) of the Centers for Disease Control and Prevention developed guidelines for the prevention and control of the spread of VRE. These recommendations were stringent and comprehensive, including prudent use of vancomycin, education of hospital staff, measures for early detection of VRE in the microbiology laboratory and immediate implementation of isolation precautions for VRE-colonised patients [33]. Although many motives favour an aggressive and maximal control of VRE, some aspects frustrate correct application of the guidelines (Table 1). Here, we discuss the clinical implications of VRE and the possibilities and dilemmas of infection control strategies.

CLINICAL IMPLICATIONS

Attributable mortality and length of stay

Despite the undisputed increased frequency of nosocomial bloodstream infections with VRE in the

Table 1. Arguments for and against the implementation of infection control measures to limit the nosocomial spread of vancomycin-resistant enterococci (VRE)

For	Against
VRE infections have attributable mortality	Associations between VRE infections and mortality only represent the severity of the underlying illness and are not attributable to vancomycin resistance per se
VRE infection increases healthcare costs	Associations between VRE infections and increased healthcare costs, again, only represent the severity of the underlying illness and cannot be attributed to vancomycin resistance per se
Infection control measures are effective in controlling the spread of VRE	Required precautions are stringent and unpleasant for patients and healthcare workers, only successful if implemented on a large scale, and the cost-efficacy of infection control measures has not been demonstrated
Infection control measures can be targeted to specificgenogroups of VRE that can be recognised phenotypically (on antibiotic susceptibility profiles) and genotypically	The existence of a large reservoir of VRE amongst healthy subjects precludes the implementation of large-scale infection control policies
VRE are a source of *vanA* for *Staphylococcus aureus*	New and effective antibiotics will be developed in time

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USA, the clinical significance of enterococcal bacteraemia remains controversial [1]. Some have suggested that VRE bacteraemia is not an independent risk factor for patient mortality, but simply a reflection of the severity of the illness. Indeed, multiple risk factors, mostly related to the severity of illness, for colonisation and infection with VRE have been identified, including increasing Acute Physiology and Chronic Health Evaluation (APACHE) II score [18,34], extensive antibiotic treatment [5,11,14,18,25,27,35–43], renal failure [5,34,38,44], prolonged hospitalisation [11,25,34,36–38,40,42,43,45], comorbidity [14,41,46] and proximity to a VRE carrier [13,14,35].

Unequivocal proof of attributable mortality of a certain nosocomial infection is difficult to establish. For VRE, most analyses evaluating attributable mortality rates have been retrospective with different study designs. In nine of the 15 studies listed in Table 2, no differences in clinical outcome were found between patients infected with VRE and patients infected with vancomycin-susceptible enterococci (VSE) [10,18,36,43,44,50], whereas negative impacts on survival in patients with VRE bacteraemia were found in six studies [5,34,45,49]. The small sample sizes, choice of comparators and differences in matching criteria may explain this discrepancy. Furthermore, differences in enterococcal species and susceptibility to ampicillin may also affect mortality risk. *E. faecium*, frequently resistant to ampicillin, currently is most often associated with nosocomial bacteraemia and has been associated with higher mortality than *E. faecalis* [5,24].

It may be concluded that VRE bacteraemia is indeed a marker for severe illness, but is less clear if vancomycin resistance in itself is associated with attributable mortality.

Nevertheless, VRE bacteraemia was generally found to be associated with excess length of stay, ranging from 6 to 22 days (Table 2).

Healthcare cost

If the length of stay in an ICU or hospital, the intensity of therapy and the expenses for antimicrobial therapy are indeed increased for patients with VRE, the attributable cost of infection with these organisms is likely to be high. Moreover, the presence of resistant microorganisms may necessitate implementation of infection control procedures, which will also increase healthcare costs [12,29,51–53]. Unfortunately, little is known about healthcare costs related to infections caused by antibiotic-resistant microorganisms or about the cost-effectiveness of infection control measures.

In head-to-head comparisons of patients infected or colonised with VRE and patients infected with VSE, VRE carriage and bacteraemia were indeed associated with higher healthcare costs and prolonged hospitalisation [40,42,48,49,51]. However, obvious associations between the severity of the underlying illness, prolonged length of stay and increased risks of acquiring colonisation or infection with resistant microorganisms may easily lead to uncertainty. For example, patients with VRE infections were associated with attributable ICU costs when compared with patients without enterococcal infections, but no such differences were found when patients with VRE infections were compared with patients with VSE infections [47]. In another study, VRE infections, when compared with VSE infections, were associated with increased hospitalisation costs for less severely ill patients, but costs were similar for VRE and VSE infections in patients with severe underlying illness [38].

The implementation of infection control measures is costly, and unequivocal proof of cost-effectiveness would be a strong argument for the widespread use of such strategies. An infection control programme including twice-weekly rectal surveillance for the early identification of critically ill patients with VRE colonisation, followed by isolation, appeared to be cost-effective [51]. However, as for methicillin-resistant *Staphylococcus aureus* (MRSA) [54], cost-effectiveness may only be applicable to high-prevalence situations, and the costs of a 'surveillance–identification–isolation' strategy relative to the costs of infections averted should be used to determine the threshold of VRE prevalence for the institution of routine surveillance [51]. Whether the prevention of endemicity of VRE in a hospital by periodical point-prevalence studies in high-risk wards, such as haemodialysis units and ICUs, is cost-effective remains to be determined.

Therapy

Enterococci are intrinsically resistant to multiple antimicrobial agents, whereas other antibiotics, such as vancomcyin, are not bactericidal at clin-

Table 2. Studies of attributable mortality and length of stay of infections with vancomycin-resistant enterococci (VRE)

Reference	Setting	Case	Comparator	Study design	Mortality difference (VRE-comparator)	Difference in length of stay (LOS) (VRE comparator)
Bhavnani *et al.* [5]	Nationwide multicentre	VRE bacteraemia	VSE bacteraemia, matched by calendar date and enterococcal species	Retrospective Matched case–control	25%	NA
Krcmery *et al.* [10]	Multicentre	VRE bacteraemia	VSE bacteraemia	Retrospective Case–control	No	NA
Mainous *et al.* [11]	Surgical ICU	VRE bacteraemia	VSE bacteraemia	Retrospective Case–control	No	16 days
Shay *et al.* [18]	Tertiary care hospital	VRE bacteraemia	VSE bacteraemia	Retrospective Case–control	No	NA
Vergis *et al.* [34]	Multicentre	VRE bacteraemia	VSE bacteraemia	Prospective observational	Yes (OR = 2.1)	NA
Garbutt *et al.* [36]	Tertiary care hospital	VRE bacteraemia	VSE bacteraemia	Retrospective Case–control	No	NA
Webb *et al.* [38]	Tertiary care hospital	VRE infection	VSE infection	Retrospective Case–control	No	NA
Stosor *et al.* [42]	Tertiary care hospital	VRE bacteraemia	VSE bacteraemia	Retrospective Case–control	25%	NA
Lucas *et al.* [43]	University hospital	VRE bacteraemia	VSE bacteraemia	Retrospective Case–control	28%	NA
Lautenbach *et al.* [44]	University hospital	VRE bacteraemia	VSE bacteraemia	Retrospective Case–control	No	NA
Linden *et al.* [45]	Liver transplant ward during outbreak of VRE	VRE bacteraemia	VSE bacteraemia	Prospective case-finding and retrospective controls	22%	Yes
Pelz *et al.* [47]	Surgical and medical ICU	VRE infection	VSE infection and non-infected	Prospective cohort	No	No
Song *et al.* [48]	University hospital	VRE bacteraemia	Controls without VRE bacteraemia matched for severity of illness	Retrospective Matched case–control	30%	17 days
Carmeli *et al.* [49]	Tertiary care hospital	VRE infection	Controls matched for ward, calendar date and duration of hospital stay at time of matching	Retrospective Matched case–control	9%	7 days
Wells *et al.* [50]	Surgical patients in university hospital during outbreak of VRE	VRE bacteraemia	VSE bacteraemia	Prospective cohort	No	NA

ICU, intensive care unit; NA, not analysed; OR, odds ratio; VSE, vancomycin-susceptible enterococci.

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ically achievable concentrations. *E. faecium* is typically resistant to high-level penicillin and ampicillin, and this type of resistance has been reported as a significant predictor of therapy failure [2,3,55]. Combination therapy of a cell-wall agent plus an aminoglycoside has become the standard of care for patients with enterococcal endocarditis, but prevalences of high-level resistance for aminoglycosides and ampicillin are increasing [2,3,5,55], leaving glycopeptides as the remaining class of active antibiotics.

Obviously, the emergence of enterococci with high-level resistance to glycopeptides has further complicated therapeutic options. Beneficial effects of chloramphenicol and tetracycline in the treatment of VRE bacteraemia have been reported, but the development of resistance during treatment has also been documented [11,56,57]. Nitrofurantoin is active against many strains of VRE, but its use is limited to urinary tract infections [55]. Ciprofloxacin is bacteriostatic for enterococci and, in combination with ampicillin or gentamicin, bactericidal *in vitro.* However, in-vivo data are lacking [55]. The newer quinolones, such as moxifloxacin, clinafloxacin and sparfloxacin, possess better activity than ciprofloxacin against enterococci and may offer additive value in the case of VRE infections [58,59].

Two classes of antibiotic have been approved specifically for the treatment of VRE infections: streptogramins and oxazolidinones [60]. Quinupristin/dalfopristin is a novel, injectable streptogramin antibiotic with in-vitro bacteriostatic activity against most Gram-positive bacteria. However, the MIC_{90} for *E. faecalis* (16 mg/L) exceeds the maximum achievable serum concentrations of 11–12 mg/L, making quinupristin/dalfopristin inactive for *E. faecalis* [61].

Reported rates of quinupristin/dalfopristin resistance amongst *E. faecium* (also resistant to vancomcyin) range from less than 10% [17,61] to 22% [34]. In addition, the emergence of resistance in *E. faecium* has been documented during therapy [17,61–63]. Clinical response rates with quinupristin/dalfopristin were 60–70% in patients with bacteraemia. Clinical failures were more common in patients with VRE endocarditis and meningitis [37,61,64].

Linezolid belongs to the oxazolidinone antibiotics and binds to the 23S ribosomal RNA of the 50S subunit on the bacterial ribosome, thereby interrupting protein synthesis [65]. Linezolid has potent in-vitro activity against both vancomycin-resistant *E. faecalis* and *E. faecium* and good therapeutic efficacy for VRE bacteraemia in mice [62]. Because of its high bioavailability, it can be administered orally and parenterally in equal doses [65]. The emergence of resistance has been reported, especially in patients who receive prolonged courses of therapy. Susceptibility testing has therefore been recommended [65,66].

The optimal antimicrobial therapy for VRE infections is not yet known. In general, antibiotics are selected according to demonstrated in-vitro activity. Most vancomycin-resistant *E. faecalis* and some *E. faecium* isolates are susceptible to ampicillin. Therefore, ampicillin, combined with gentamicin in the case of endocarditis, remains the recommended therapy for infections caused by such organisms. Although enterococci with MIC values of 16 mg/L for ampicillin are considered to be resistant according to NCCLS guidelines, it has been suggested that the combination of high-dose ampicillin (18–30 g/day) and an aminoglycoside could be effective in patients with endocarditis or persistent bacteraemia caused by VRE strains with MIC values for ampicillin of up to 64 mg/L [19].

INFECTION CONTROL ISSUES

Detection

Clinical cultures with VRE only represent the tip of the iceberg. It has been estimated that, for every patient with VRE isolated from clinical cultures, at least 10 other patients will be colonised [67,68]. Asymptomatic carriers may remain unnoticed for long periods of time, facilitating the widespread dissemination of VRE within populations. As enterococci are not considered gastrointestinal pathogens, focused surveillance for selective isolation is necessary to determine the total size of the iceberg.

Several microbiological screening methods have been developed for VRE using stool specimens, rectal swabs or perirectal swabs. Isolation of VRE from heavily contaminated specimens, such as faeces, can be hampered because the growth of VRE is inhibited by the indigenous intestinal flora, as has been shown in an in-vitro anaerobic continuous-flow culture model [69]. Nevertheless, isolation rates of VRE from stool specimens are generally higher than those from

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rectal swabs [67,70] and, in one small study, perirectal swabs were equally sensitive as rectal swabs for the detection of VRE colonisation [71].

Direct inoculation of rectal swabs on vancomycin-containing agar plates for selective cultivation of VRE has been used in many laboratories [72]. The use of solid media instead of broth offers the advantage of a rapid assessment of colony morphology but, unfortunately, low densities of VRE may not be detected [67]. Detection can be optimised by inoculating faecal samples or swabs in broth enrichment, such as Enterococcosel broth with vancomycin and aztreonam, followed by subculture on agar plates containing vancomycin. This method is, of course, more time-consuming, but has been recommended for surveillance cultures from patients [67,70,73–75] and environmental surfaces [76]. In one study, however, no advantage was found with enrichment compared with direct plating [77].

The concentration of vancomycin in selective media directly influences the specificity and sensitivity; low concentrations (4–6 mg/L) have low specificities due to the co-isolation of vancomycin-intermediate susceptible enterococci and non-enterococcal species, such as lactobacilli [70,72,74]. Higher concentrations (≥ 16 mg/L) may result in suboptimal detection of *vanC*-resistant enterococci, without masking the detection of *vanA*-resistant enterococci [72].

However, even with all these considerations, the detection of VRE may remain problematic. Obvious relapses of colonisation have been reported frequently and are at least partly due to the limited sensitivity of VRE culture methods [67,78]. 'False negative' culture results may reflect a decrease in the quantity of VRE to an undetectable level rather than true eradication. Such patients could, unjustly, be considered as non-colonised. However, undetected faecal colonisation may still result in skin contamination, and subsequent dissemination via person-to-person contact within hospitals [16,67]. High rates of false negative results with direct plating methods could be overcome by repeated sampling [72]. HICPAC guidelines recommend that a previously colonised patient can be considered to be non-colonised after three consecutive negative cultures obtained with at least a 1-week interval [33]. However, even three consecutive negative cultures do not unequivocally prove the eradication of VRE [79–81]. Therefore, guiding infection control strategies on the criteria of three consecutive negative cultures may lead to false interpretation of the true colonisation status of a patient.

Sources and transmission

VRE in the USA

There is huge inter-hospital variation in the prevalence of VRE worldwide, although most infections and hospital outbreaks have occurred in the USA. There, hospitalised patients, especially those treated in high-risk wards, such as ICUs and haemodialysis wards, should be considered as the largest reservoir of VRE. Recent studies, however, have clearly shown that the epidemiology of VRE is no longer restricted to hospital settings, as patients from long-term care facilities appear to be colonised with VRE with increasing frequency [82–84]. Colonised residents of long-term care facilities may therefore become important VRE reservoirs and pose a potential infection control risk when admitted to an acute care hospital.

The gastrointestinal tract and skin are the most important sites of VRE colonisation. Intestinal colonisation almost always precedes VRE bacteraemia [51,79,85], and appears to be the major reservoir from which the spread of the organism occurs in healthcare settings [78–80,86–89]. Skin colonisation may also contribute to the dissemination of VRE and can be a source of intravascular device-associated infections [67,85].

Temporarily contaminated hands of healthcare workers are important vectors for the transmission of VRE amongst patients [50]. The process of cross-transmission requires healthcare workers to have contact with different patients, some of whom are colonised with VRE, and involves lapses in infection control. Therefore, contact rates (or workload), the likelihood of contact with already colonised patients (colonisation pressure), the likelihood of subsequent contact with a non-colonised patient and adherence to infection control measures (most notably hand disinfection) are important variables in the dynamics of colonisation [90].

The duration of hospitalisation, admittance to an ICU and antibiotic use have been associated with prolonged carriage of VRE [80,91]. Not all antibiotic classes are equally effective in selecting for VRE. Vancomycin and cephalosporins have

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been selected as independent risk factors in many studies. In addition, treatment with anti-anaerobic antibiotics has been repeatedly associated with high-density VRE colonisation [67,92]. Interestingly, vancomycin has repeatedly been identified as a risk factor for VRE colonisation. However, selecting the comparator in such analyses is crucial [93]. In a meta-analysis, analysing the risks of vancomycin therapy for VRE colonisation and infection, the pooled odds ratio (OR) for vancomycin use was 10.7 for studies that employed a control group of patients with VSE colonisation [94]. Studies using control patients who were not limited to VSE colonisation revealed a much weaker association (OR, 2.7), which was completely eliminated when the analysis was limited to studies that also controlled for the time at risk prior to the outcome. The explanation for this bias is that treatment with active antibiotics (such as vancomycin for VSE) probably inhibits the growth of susceptible bacteria, thereby making exposure less frequent in patients colonised with susceptible bacteria, and thus overestimating the risk.

The eradication of VRE colonisation has not (yet) been possible, although a combination of oral bacitracin and doxycycline showed transient efficacy [95,96]. In addition, suppression of gastrointestinal colonisation was achieved with ramoplanin, although colonisation rates had completely recovered by day 21 after the discontinuation of therapy [97].

Sustained VRE colonisation amongst healthy American subjects remains extremely rare, although colonisation has been demonstrated in the gastrointestinal tract of healthcare workers and family members of patients [98]. In one study, three patients admitted directly from the community, and with no history of recent previous hospitalisation, appeared to be colonised with VRE upon admission. These findings suggest that VRE colonisation may have persisted for long periods of time after remote nosocomial acquisition, or that VRE colonisation had been acquired from unidentified community sources [99]. Acquisition of VRE amongst healthy individuals in the community, without previous hospitalisation, has only been observed once [100]. Therefore, in the USA, reservoirs of VRE are present predominantly in healthcare facilities and a significant community reservoir of VRE has not been demonstrated.

In addition to patients themselves, their inanimate contaminated environment may play a role in the epidemiology of VRE. Contamination of environmental surfaces or equipment has been documented frequently in clinical and out-patient settings [53,83,84,101–104]. Contact with such surfaces may contaminate hands [105]. However, the relative importance of environmental contamination in epidemiology is unknown. In one study, contamination occurred frequently, but usually temporarily and with low bacterial counts, suggesting that the environment was more a recipient than a source for patient colonisation [106]. Nevertheless, contamination of a blood pressure cuff by one patient, followed by acquired colonisation of the next patient treated in that room with the same genotype of VRE, was shown in the same study [106]. The exact role of environmental contamination in epidemiology, and thus the necessity to include enforced environmental cleaning as an infection control measure, has not been determined.

VRE in Europe

The European epidemiology with respect to VRE is almost completely opposite to the American situation. In Europe, only a few nosocomial outbreaks have been reported (although this incidence is increasing) and incidences of nosocomial infection are low. However, asymptomatic carriage amongst healthy European individuals is relatively common [12,37,107–112]. This large reservoir of VRE amongst healthy subjects has been associated with the prolonged use of the vancomycin analogue, avoparcin, as a growth promoter in the livestock industry. Indeed, VRE colonisation rates are extremely high in pigs, calves and turkeys [109,111–113], and VRE has also been found, although at much lower rates, in the faeces from cats and dogs [114,115], shellfish [116], woodmice and badgers [117]. Individuals in close contact with these animals showed higher rates of VRE colonisation than did healthy subjects without such contacts. In addition, genotypically identical transposons containing the *vanA* gene have been demonstrated in enterococci from animals and farmers, slaughterers and residents [109]. The link between avoparcin use and the existing community reservoir of VRE is further supported by the absence of VRE colonisation amongst farm animals and healthy citizens in the USA, where glycopeptides have never been used

as growth promoters for livestock [100,118,119]. Moreover, after the ban on avoparcin use in the agricultural industry in the European Community in 1997, the prevalence of VRE in broiler chickens and healthy persons decreased in several European countries, including the Scandinavian countries, the Netherlands and Germany [120,121]. Of note, however, are the high prevalences of VRE which persisted in broiler and turkey carcasses in Denmark and Norway after the ban [121,122], as well as in Swedish sewage samples [123]. Whether the ban on avoparcin use in the agricultural industry will completely eliminate the community reservoir of VRE in Europe is not yet known. All of these associations strongly suggest a causal relationship between the antibiotic-driven selection of VRE in animals and subsequent spillover into the human population. How should this linkage be interpreted in view of the differences between European and American epidemiology?

Molecular genotyping using amplified fragment length polymorphism and multilocus sequence typing has identified different genogroups of VRE amongst humans and animals [124]. Interestingly, vancomycin-resistant *E. faecium* isolates from pigs and chickens belonged to a genetically identical cluster as isolates from non-hospitalised humans [110,125], supporting the transmission of strains from animals to humans. Remarkably, strains recovered from hospitalised patients and hospital outbreaks in the USA, Europe and Australia belonged to a distinct genetic lineage of *E. faecium* [110,125]. Isolates belonging to this lineage were characterised by ampicillin resistance and the presence of the variant *esp* gene, encoding for an enterococcal surface protein. Both characteristics are almost completely absent in non-epidemic human and animal isolates [125]. These preliminary findings suggest that a clonal lineage of *E. faecium*, capable of surviving in hospital settings, and selected because of resistance to ampicillin and vancomycin, has emerged on several continents. If true, this would at least partly explain why VRE has emerged in American hospitals without the presence of a community reservoir.

Evaluation of infection control measures

The HICPAC guidelines for the prevention and control of the spread of VRE focus on the prudent use of vancomycin, education of hospital staff, early detection of VRE in the microbiology laboratory and immediate implementation of isolation precautions [33].

Several studies have confirmed the effectiveness of the complete package of measures in controlling nosocomial outbreaks [14,37,126,127], and even in reducing endemic prevalence [30], although complete eradication of VRE from a hospital appears to be extremely difficult [29]. Moreover, an active infection control programme, including surveillance cultures and the isolation of infected patients, reduced the overall prevalence of VRE in 32 healthcare facilities in three US states in 3 years from 2.2% to 0.5% [128]. Enhanced infection control measures, such as weekly surveillance cultures, observation of hand-washing practices and cohorting of patients and nurses, also decreased the incidence of VRE bacteraemia, antibiotic use and healthcare costs [51,129]. However, despite the formulation of guidelines and reports of successful interventions, the incidence of VRE infections is still increasing in the USA [1]. A simple explanation is that, despite all the guidelines, endemicity persists because of poor hand hygiene compliance [29]. It may also be argued that control measures have been implemented too late; the existing reservoir of VRE within hospitals is now too large for successful infection control. Moreover, the extramural population of VRE carriers, especially those situated in long-term care facilities, may have created such a large influx of VRE [130] to render even improved infection control measures ineffective. It is also possible that transmission occurs via as yet unidentified routes that are insufficiently addressed in the current guidelines. For example, long-term environmental contamination could serve as a constant reservoir for transmission, and thus complete prevention of patient-to-patient transmission would still not lead to a decrease in colonisation originating from environmental surfaces.

The level of infection control measures required depends on the number of patients admitted with VRE, their length of stay and the baseline level of endemic prevalence. Increases in these variables can only be compensated for by large increases in barrier precautions. Mathematical analysis of VRE endemicity within an ICU setting has suggested that, considering the epidemiological dynamics in this setting, a mean endemic prevalence of 75% will be

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established if no infection control measures are implemented. Owing to infection control measures (such as 50% compliance for hand disinfection and a relatively high cohorting level of nurses to individual patients), the observed mean endemic prevalence was found to be 36% [90]. According to this model, infection control therefore reduced the endemic prevalence from 75% to 36%.

The relative importance of individual infection control measures has not been determined, although the effects of individual measures have been evaluated in some studies.

Reducing the antibiotic pressure seems to be a logical approach for the control of VRE, as multiple studies have identified antibiotic therapy as a risk factor for acquisition. Changes in specific prescriber practice in ICUs were associated with decreased vancomycin use and VRE prevalence, when compared with ICUs in which no unit-specific changes had been implemented [131]. In contrast, VRE prevalence increased steadily over a 10-year period, despite dramatic reductions in cephalosporin use, without concomitant aggressive infection control interventions [132]. A programme combining the restricted use of vancomycin, cephalosporins and clindamycin and the use of gowns for VRE-colonised patients was associated with a considerable decrease in VRE prevalence [133].

The use of gowns in addition to gloves is controversial. The use of gloves and gowns helped to control a monoclonal VRE outbreak [134], and protected against the acquisition of VRE, especially in patients with long-term VRE exposure [135]. In contrast, the universal use of gloves and gowns was no better than glove use only in preventing colonisation with VRE in an endemic ICU setting [68]. Part of the success of using gowns may, in fact, result from better compliance with other infection control procedures when gowns are required, probably because of enhanced awareness of transmission dynamics [135].

Transfer of *vanA* to *Staphylococcus aureus*

Vancomycin is a crucial antibiotic for infections caused by MRSA. Ever since enterococci developed plasmid-based resistance to vancomycin, the genetic transfer of vancomcyin resistance to MRSA has been feared. Indeed, the transfer of the *vanA* gene from *E. faecalis* to *S. aureus* in colonised mice was demonstrated in 1992 [136]. However, until 2002, decreased susceptibility to vancomycin in *S. aureus* was restricted to so-called vancomycin-intermediate *S. aureus* isolates based on the thickening of the staphylococcal cell wall, with MIC values of 8 mg/L [137].

The first isolate of vancomycin-resistant *S. aureus* (VRSA) harbouring the *vanA* gene was recovered from a catheter exit site and a foot ulcer from a patient with diabetes, peripheral vascular disease and chronic renal failure in 2002 in Michigan, USA [138,139]. In addition to VRSA, vancomycin-resistant *E. faecalis* was cultured from the foot, and vancomycin-susceptible MRSA had been identified in earlier cultures. The patient had been treated with multiple courses of antibiotics, including vancomycin. The isolate contained the enterococcal *vanA* gene, consistent with the vancomycin MIC profile of 32 mg/L, and the *mecA* gene, which confers resistance to oxacillin. The DNA sequences of *vanA* genes from VRSA and VRE isolates were identical, and vancomycin-susceptible MRSA and VRSA isolates were indistinguishable by pulsed-field gel electrophoresis, strongly suggesting that the *vanA* gene had jumped from the enterococcal donor strain to the patient's MRSA strain [138]. The second clinical isolate of VRSA was isolated a few months later from a patient admitted to a hospital in Pennsylvania, USA [140]. This second case appeared to be epidemiologically unrelated to the first case and, as both strains probably resulted from conjugation events, additional VRSA infections are to be expected.

CONCLUSION

VRE have emerged as important nosocomial pathogens, initially in the USA, but hospital outbreaks are also being reported with increasing frequency in Europe. Reasons for the need to control the spread of VRE include the probable attributable mortality and increased healthcare costs due to VRE infection. Nevertheless, the incidence of VRE infections is still extremely low in most European countries, and therefore the need for control must be balanced against the costs associated with the implementation of effective strategies. However, the recently demonstrated in-human transmission of vancomycin resistance from VRE to MRSA underscores the

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potential danger of a coexisting reservoir of both pathogens. As MRSA is already endemic in many European hospitals, the prevention of VRE endemicity seems to be the only alternative.

The presence of a large community reservoir of VRE in Europe could hamper the feasibility of infection control strategies. Based on prevalence studies, up to 5% of non-hospitalised humans could be asymptomatically colonised with VRE in some European countries. Although this percentage is apparently decreasing after the ban on avoparcin use in the agricultural industry, it still means that a large proportion of admitted patients are potential sources for VRE transmission. With no risk profile available to identify these carriers, effective screening, followed by barrier precautions for carriers, will simply be impossible. Recent studies, however, suggest that hospital outbreaks are almost exclusively caused by a specific genogroup of vancomcyin-resistant *E. faecium* that can easily be characterised by co-resistance to ampicillin and the presence of the variant *esp* gene [125].

Based on our own experience, we propose that VRE infection control programmes should be restricted to patients colonised by *esp*-positive VRE strains. Ampicillin resistance appears to be a specific and sensitive marker for this genogroup [141]. If such a strain is cultured from a clinical sample, surveillance amongst contact patients, in order to trace an outbreak as soon as possible, is recommended. For the same reason, it may be wise to perform point-prevalence studies, periodically, on high-risk wards, such as ICUs and haemodialysis wards. We suggest that patients who are found to be colonised with such strains should be marked in the hospital information system. Isolation precautions for these patients should be continued, even when surveillance cultures have become negative for VRE. As these patients are often transferred between healthcare facilities, national and, possibly, international guidelines to control the spread of VRE should be established.

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