

Vancomycin-resistant Enterococci

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"Common conditions occur commonly; uncommon conditions occur less commonly. Uncommon presentations of common conditions are commoner than common presentations of uncommon conditions."-adapted from a Singapore professor

Abstract

Vancomycin-resistant enterococci (VRE) are gaining much attention in the West, chiefly because of the lack of available antimicrobial therapy for VRE infections as most VRE are also resistant to drugs previously used to treat such diseases (e.g. aminoglycosides and ampicillin), the possibility that the vancomycin-resistant genes present in VRE can be transferred to methicillin-resistant Staphylococcus aureus (MRSA), and increasing reports of VRE and the trend towards endemicity in North America. There are three case reports and a study showing stool carriage of more than 10% from Singapore. One of the case reports is notable as the VRE isolated from the urinary tract is community-acquired. In Europe, there is a strong association between the use of avoparcin (a glycopeptide) in animal feeds and the emergence of VRE. Clonal dissemination resulting in nosocomial transmission is also demonstrated. Prior vancomycin use is a risk factor for the subsequent development of VRE bacteraemia. The laboratory plays an important role, namely, a) detection of VRE, and b) determination of susceptibilities of antimicrobials whereby possible therapeutic options may be instituted when antimicrobial intervention is indicated. There is a need to evaluate existing infection control measures against VRE to prevent it from becoming endemic in Singapore as had happened in North America.

Ann Acad Med Singapore 1997; 26:808-14

Key words: Enterococci, Review, Singapore, Vancomycin resistance

Introduction

Since 1989 a rapid increase in the incidence of infection and colonisation with vancomycin-resistant enterococci (VRE) has been reported in hospitals in the United States.¹ The problems posed by VRE are as follows:

- a) the lack of available antimicrobial therapy for VRE infections as most VRE are also resistant to drugs previously used to treat such infections (e.g. aminoglycosides and ampicillin),²
- b) the possibility that the vancomycin-resistant genes present in VRE can be transferred to other gram-positive microorganisms [e.g. methicillin-resistant Staphylococcus aureus (MRSA)],³ and
- c) the rapid increase of reports of VRE.¹

Growing Concerns in the West over VRE

Vancomycin-resistant *Enterococcus* was first isolated in Europe in the second half of the 1980s,^{4,5} and currently appear to be an alarming problem especially in the United States.⁶ It is the most rapidly increasing antibiotic-resistant bacteria reported by hospitals in New Jer-

sey.⁷ In New York, the number of hospitals reporting VRE increased from 1 in 1989 to 38 by October, 1991.⁸ National Nosocomial Infections Surveillance conducted by the Communicable Disease Centre demonstrated a 20-fold increase in the percentage of enterococci associated with nosocomial infections that are resistant to vancomycin from 1 January 1989 to 31 March 1993.⁹

What about Singapore?

Unlike the West, VRE has yet to become a major problem in Asia with the sole exception of Taiwan. A recent seminar "Regional Seminars on Bacterial Resistance" held in Singapore from 13 to 15 December 1995 reported the incidence of VRE in Taiwan and China to be 30% and 7%, respectively. To date in Singapore, VRE has been isolated in only 3 cases: the first in a wound of a burns patient in the Singapore General Hospital isolated in 1994¹⁰ and the other two from urinary specimens from the Tan Tock Seng Hospital in 1995.¹¹ The two patients with VRE urinary tract infections did not have any prior antimicrobial therapy while the burns patient had courses of penicillin and gentamicin but not vancomycin. The

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VRE isolated from the urinary tract of one of the patients was “community-acquired” which brings into question the source of VRE acquired in the community. A recently completed study carried out in 1997 at the National University Hospital revealed that more than 10% of patients carried enterococci with a decreased susceptibility to vancomycin in their faeces.¹² Half of these patients did not have any prior antimicrobial therapy. Indeed, VRE is also emerging in Singapore and Asia.

Hospital Transmission of VRE

Bonten et al¹³ investigated colonisation of VRE in patients and environmental contamination with VRE in a medical intensive-care unit (MICU), an endemic area in one of the hospitals in the United States. Their aim was to assess the importance of different sources of colonisation. Cultures were taken from the following body sites: rectum, groin, arm, oropharynx, trachea, and stomach. The environmental surfaces cultured included bedrails, drawsheet, blood-pressure cuff, urine containers, and enteral feed. Bonten et al found that frequent colonisation with VRE in the MICU and subsequent cross-colonisation were important factors in the endemic spread of VRE. Persistent VRE colonisation in the gastrointestinal tract and on the skin, the presence of multiple-strain types of VRE, and environmental contamination contributed to the spread of VRE. Fingertips were also common sites for colonisation especially if gloves were not worn and proper handwashing was not carried out.¹⁴ Cross-colonisation of enterococci in the process of clonal dissemination was demonstrated by the author using three different molecular typing techniques: randomly amplified polymorphic DNA polymerase chain reaction method, restriction fragments length polymorphism by pulse field gel electrophoresis and conventional gel electrophoresis.¹⁵ These findings were also observed by others working on enterococci.⁸

Risk Factors Associated with VRE

VRE infections are typically seen in hospitalised patients with at least one co-morbid condition¹⁶ (Table I). Nosocomial outbreaks have been reported from medical-surgical ICUs,^{17,18} oncology units,¹⁹ among patients with renal failure on haemodialysis,⁵ and among organ transplant recipients.²⁰ Prior vancomycin use and / or cephalosporin was demonstrated to be a risk factor for the subsequent development of VRE bacteraemia.^{8,20,21} Kaplan et al²¹ reported an increased incidence of stool colonisation with VRE following vancomycin use. These data are particularly relevant since stool colonisation may be a precursor of invasive VRE infection.¹⁸

A review analysing the clinical outcome of 96 evaluable patients derived from different reports²⁰ found that the overall mortality was 55.2% (range 28% to 100%). Studies have reported an increased mortality of patients

TABLE I: RISK FACTORS FOR VRE INFECTION†

Advanced age
Severe underlying illness
Immunosuppression
ICU residence
Prior hospitalisation
Surgery (especially gastrointestinal, cardiothoracic and transplantation)
Antibiotic exposure
Third-generation cephalosporin
Vancomycin use
Antibiotics effective against anaerobic organisms
Invasive devices

Data are obtained from ICUs, oncology units, dialysis units and organ transplant units.

† Adapted from: Joshi N, Milfred D, Caputo G. Vancomycin-resistant enterococci: a review. *Infect Dis Clin Prac* 1996; 5:528-37.

with VRE bacteraemia compared with that of control patients with vancomycin-sensitive enterococcal bacteraemia.^{1,22}

Animals are associated with VRE transmission

Shortly after the first report about the natural occurrence of vancomycin-resistant *Enterococcus faecium* possessing the *vanA* gene-mediated resistance mechanism, numerous outbreaks caused by VRE had been recorded in a variety of clinical settings. Although not all of the VRE isolates from these outbreaks were subjected to molecular typing, there are examples for both exogenous infections by spread of a particular strain, presumably related to poor hospital hygiene,^{8,17} and endogenous infections as indicated by different typing patterns of corresponding enterococcal isolates.²³ The latter findings suggest that VRE has spread among the community and may have become part of the intestinal flora of patients entering hospitals.²⁴ Widespread VRE outside hospitals was already established by isolation from sewage treatment plants.^{25,26} The finding of VRE in a sewage treatment plant in a rural area of former East Germany with only exceptional and rare use of glycopeptides in the local hospital?” suggested that hospitals may not be the only source of VRE, and that other centres of selective pressure had to be looked for. Besides their use in human chemotherapy, glycopeptides are also used as growth promoters in animal husbandry. Since the late 1970s, the glycopeptide avoparcin (Avotan, Cyanamid International) has been available as a feed additive in many countries (although not in the United States and Canada). Selective pressures in favour of glycopeptide resistance should be critically evaluated, not only in human chemotherapy but also urgently in animal husbandry.²⁴

Genetics of Glycopeptide Resistance in Enterococci

The glycopeptide antibiotics, vancomycin and

teicoplanin, inhibit the late stages of cell wall synthesis by forming a complex with the C-terminal D-alanyl-D-alanine (D-Ala-D-Ala) residues of peptidoglycan precursors.²⁷ These target residues are synthesised as a dipeptide D-Ala-D-Ala in the cytoplasm by a D-Ala:D-Ala ligase and are added to UDP-*N*-acetylmuramyl-L-Ala-γ-D-Glu-L-Lys by a D-Ala-D-Ala adding enzyme.

Glycopeptide resistance in enterococci is phenotypically and genotypically heterogeneous.²⁸ These features are summarised succinctly in Table II. The genes responsible for inducible resistance to high levels of vancomycin and teicoplanin (VanA phenotype) are carried by the 10 851-bp Tn1546 transposo.²⁹ Transposition of Tn1546 into self-transferable plasmids and subsequent transfer by conjugation appears to be responsible for the dissemination of this type of resistance.³⁰ Nine polypeptides are encoded by Tn1546 that belong to five functional groups:³⁰ transposition functions (ORF1 and ORF2), regulation of resistance gene expression (VanR and VanS), synthesis of modified peptidoglycan precursors (VanH and VanA), hydrolysis of D-Ala-D-Ala-containing peptidoglycan precursors (VanX and VanY), and low-level teicoplanin resistance (VanZ). VanB-type resistance (various levels of resistance to vancomycin and susceptibility to teicoplanin) is also due to production of D-Ala-D-Lac. The VanB ligase of VanB-type strains is structurally and functionally similar to VanA.³¹ The VanB gene was found on composite transposon Tn1547, which in turn, was part of larger conjugative chromosomally located elements (90 to 250 kb). In contrast to acquired VanA- and VanB-type resistance, VanC-type resistance (low level of resistance, to vancomycin and susceptibility to teicoplanin) is an intrinsic property of motile enterococci. Resistance in these species is due to synthesis of dipeptide D-Ala:D-Ser by VanC ligases leading to production of cell wall precursors with reduced vancomycin affinity.³²

Laboratory Detection of VRE

The success of the hospital's infection control efforts over VRE whether nosocomial or community-acquired hinges to a large extent on the active involvement of the laboratory in all aspects of the infection control programme. Laboratory personnel should understand why infection control is necessary, the approaches being taken by the hospital's infection control programme to meet its objective in reducing nosocomial infections, and how the laboratory can support and cooperate in the programme.

The methods available in the rapid screen for VRE include disk diffusion tests and agar or broth methods. Initial problems with detection of resistance using disk diffusion led to recommendations of new diffusion breakpoints.³³ It is recommended that any haze or colonies within the zone be taken into account when determining zones of inhibition and that a minimal inhibition concentration (MIC) test be performed for strains with intermediate zones if vancomycin is considered for treatment. Examples of rapid agar and broth screen methods are: kanamycin-aesculin azide enrichment broth supplemented with vancomycin 20 mg/L,³⁴ brain heart infusion agar supplemented with vancomycin 6 mg/L,³⁵ Campylobacter blood agar with clindamycin and vancomycin 10 mg/L,³⁶ enterococcosel broth with vancomycin 6 mg/L,³⁷ and brain heart infusion broth supplemented with vancomycin 4 mg/L, amphotericin 2 mg/L and aztreonam 4 mg/L.³⁸ The broth methods require further subculturing onto agar plates in order to isolate the colonies. Colonies are specifically identified as enterococci by the pyroglutamyl-beta-naphthylamide (PYR) and bile esculin tests. Further testing using API 20 Strep (Bio Merieux SA, France) confirms the genus and identifies the species. Comparison of pigment production and motility tests together with API 20 Strep kit are sufficient for identifications of *E. faecium*, *Enterococcus*

TABLE II: GLYCOPEPTIDE RESISTANCE IN ENTEROCOCCI†

Resistance	Acquired		Intrinsic
	High level	Various levels	Low Level
Phenotype	VanA	VanB	VanC
MIC (µg/ml)			
Vancomycin	64 → 1000	4 → 1000	2 - 32
Teicoplanin	16 - 512	0.5 - 1	0.5 - 1
Expression	Inducible	Inducible	Inducible constitutive
Location of R genes	Plasmids	Chromosome	Chromosome
Mobile element	Tn1546	90 - 250 kb	
Transfer by conjugation	+	+	
Microorganism	<i>Enterococcus spp.</i>	<i>E. faecium</i> , <i>E. faecalis</i>	<i>E. gallinarum</i> , <i>E. casseliflavus</i> , <i>E. flavescens</i>

† Adapted from: Evers S, Quintiliani Jr R, Courvalin P. Genetics of glycopeptide resistance in enterococci. Microbial Drug Resistance 1996; 2:219-23.

casseliflavus, and *Enterococcus gallinarum*.³⁹ The latter two species produce VanC resistance and are less reliably identified by commercial kits. Commercial vancomycin agar screen plates are also available for detection of VRE. At least five commercial sources are known: BD Microbiology Systems, Carr-Scarborough Microbiologicals, MicroBio Products, PML Microbiologicals, and REMEL. Detection of VRE ranged from 94% for BD Microbiology Systems to 99% for PML Microbiologicals.⁴⁰ Automated systems such as Vitek software version 7.1 has also being evaluated and the GPS-TA card was found to be 98% sensitive and 95% specific for the detection of vancomycin resistance, generating a total of four very major (1.6%) and five minor errors (1.3%).⁴¹ Oon and the author¹² carried out pilot experiments evaluating several methods and the following observations were made: the Swenson et al³⁵ method is not suitable as it could not suppress yeasts and gram-negative rods which are present in large numbers in stool specimens, while the Rao et al³⁸ method is cumbersome as it requires subculturing onto agar plates. We¹² modified these methods which comprise of brain heart infusion agar incorporated with vancomycin 4 mg/L, amphotericin 2 mg/L and aztreonam 4 mg/L, and enterococcosel agar with vancomycin 6 mg/L. The methods are cheap and easy to produce and the results are readily interpreted.

Other Antimicrobial Resistances

The treatment of VRE infections is a clinician's nightmare because the microorganism has multiple drug resistances. It is intrinsically resistant to cephalosporins, lincosamides, acquired resistances to tetracyclines (via genes such as *tetM* and *tetN*), the macrolides (via MLS) and chloramphenicol (via chloramphenicol acetyltransferase). Enterococci are more resistant to penicillin and ampicillin than streptococci,⁴² *E. faecium* rather more so than *E. faecalis*. Typical MIC values for penicillin and ampicillin are 2 to 4 mg/L and 0.5 to 2.0 mg/L respectively for *E. faecalis*, and 8 to 16 mg/L and 4 to 8 mg/L for *E. faecium*. Low affinity of the penicillin-binding proteins accounts for their resistances. Furthermore, enterococci are usually tolerant to these antibiotics, necessitating combination with an aminoglycoside to achieve synergistic bactericidal effect. The mechanism by which such combinations yield synergy has been shown to be the facilitation of aminoglycoside entry by the disruptive effect of antibiotics acting on the cell wall.⁴³ The presence of high-level aminoglycoside resistance (HLAR) implies loss of synergy between a cell wall active antimicrobial agent (penicillin or ampicillin) and an aminoglycoside. The determination of the HLAR phenomenon is therefore necessary to decide whether or not such a combination could be used.⁴⁴ As enterococcal resistance to gentamicin and streptomycin occur by different mechanisms, it is important to test susceptibilities to both agents. Gen-

tamicin resistance is predominantly the result of the presence of the inactivating enzyme 2'-phosphotransferase-6'-acetyltransferase conferring resistance to gentamicin, tobramycin, netilmicin, amikacin, and kanamycin, while maintaining sensitivity to streptomycin. Hence, gentamicin resistance is a good predictor of resistance of other aminoglycosides except streptomycin. Streptomycin resistance is caused mainly by the presence of streptomycin adenylyl-transferase, maintaining sensitivity to gentamicin.

Novel Agents against VRE

There is no standard therapy for VRE. Although some agents have shown *in vitro* activity alone or in combination, including ciprofloxacin, doxycycline, novobiocin, teicoplanin, chloramphenicol and rifampin, treatment options are limited to combinations of drugs with marginal efficacy against the pathogens. Quinupristin-dalfopristin (RP59500) is a new investigational agent with activity against gram-positive cocci, including VRE.⁴⁵ It is a water-soluble streptogramin composed of two semisynthetic derivatives of the main components of the oral streptogramin and pristinamycin. These are quinupristin (derived from pristinamycin I) and dalfopristin (derived from pristinamycin II), which although bacteristatic individually, exert bactericidal activity in combination against the bacteria. Quinupristin binds to the 70 S ribosome and, more specifically, to protein L₂₄ of the 50 S subunit. Dalfopristin appears to modify proteins L₁₀ and L₁₁ of the 50 S ribosomal subunit. Anecdotal reports of cure are seen in VRE causing CAPD-peritonitis,⁴⁶ shunt infection in infant⁴⁷ and aortic graft infection.⁴⁸

Other examples of novel antimicrobial agents include fluorinated oxazolidinones U-100592 and U-100766,⁴⁹ everninomicin SCH 27899,⁵⁰ and semisynthetic amide derivatives of teicoplanin, MDL 62208, MDL 62211, and MDL 62873.⁵¹

To Treat or Not to Treat VRE?

The clinical significance of positive cultures for VRE should be carefully evaluated before embarking on drug treatment. Microbial colonisation does not require treatment although there are reports of subsequent infection following colonisation especially in immuno-compromised host.⁵² Furthermore, polymicrobial infections involving the abdominal cavity of the skin and soft tissue in immunocompetent patients frequently respond to therapeutic options not particularly directly towards VRE as observed by Lai.⁵³ VRE line-related bacteraemias could be treated by line removal alone. Surgical site infections, soft tissue infections, and abscesses could be managed by surgical debridement and drainage without specific antimicrobial agents against VRE. Removal of foreign devices, debridement, and surgical drainage

seemed to be important in the resolution of VRE infections. Infections of the lower urinary tract may resolve spontaneously, by removal of Foley catheters or respond to single oral agents, like amoxicillin, nitrofurantoin, or ciprofloxacin, because of the very high levels such agents achieve in the urine, when they are susceptible microbiologically. Life-threatening infections, such as bacteraemia or endocarditis, usually require bactericidal therapy. Unfortunately, the paucity of human clinical data and poor correlation of *in vitro* and animal data to human infection necessitate the reliance on primarily anecdotal evidence. The clinical results of one group may not corroborate those from another because of different laboratory methodologies and patient populations. Caution is warranted in the interpretation of such data since clinical significance is limited by the small size and uncontrolled nature of the studies.

Based on available data, the following approach to treat serious VRE infections is suggested:¹⁶

1. If the organism retains some susceptibility to gentamicin (MIC <32 mg/L), this agent should be included as one component of a combination regimen. If no cell-wall active agent (e.g. ampicillin or teicoplanin) is available based on susceptibility testing, the combination of vancomycin and penicillin with gentamicin should be used, despite the presence of *in vitro* resistance to vancomycin or ampicillin alone. If high-level gentamicin resistance (MIC >500 mg/L) is demonstrated, this agent will no longer be effective in any regimen.
2. Ciprofloxacin may be employed in combination if the MIC is demonstrated to be <8 mg/L. Companion agents may be chosen from ampicillin, novobiocin, or rifampin. As with gentamicin, ciprofloxacin probably should not be used if high-level resistance (MIC >32 mg/L) to ciprofloxacin is documented.
3. Ampicillin or teicoplanin should be used if the isolate is susceptible, and they should be used in combination with an aminoglycoside if suggested by MIC or synergy panel data.
4. Despite low-level ampicillin resistance (MIC <32 mg/L), some benefit may still be derived as monotherapy, but if the MIC is >64 mg/L, a combination regimen should be used.
5. In recognition of its anecdotal benefit in some reports, the streptogramin, quinupristin/dalfopristin, may be considered in similar situations.
6. When other options are lacking, the use of chloramphenicol may be cautiously considered.

Infection Control, an Indispensable Tool in the Control of Nosocomial VRE

Clearly, the arguments thus far indicated that patient-to-patient transmission of microorganisms can occur either through direct contact or through indirect contact

via the hands of personnel or contaminated patient-care equipment or environmental surfaces. Recommendations of the American Hospital Infection Control Practices Advisory Committee are as follows:⁵⁴

- a) prompt notification of hospital staff and education in hospital VRE policy,
- b) establishment of a monitoring system,
- c) isolation of infected or colonised patients in single rooms or with other patients who have VRE,
- d) routine use of gloves by personnel entering the room (with gowns if there is to be substantial contact),
- e) dedication of equipment to a single patient or cohort of patients with VRE where possible,
- f) cultures of stools or rectal swabs to determine colonisation status,
- g) maintenance of isolation precautions until cultures from multiple sites are negative on three consecutive occasions, and
- h) national computerised records of colonised patients.

VRE-Common or Uncommon in Singapore?

The gastrointestinal tract is a possible reservoir for VRE.³⁴ Should more studies of stool carriage be carried out using rapid screening methods to detect VRE in other Singapore hospitals? Is there a need to screen animal imports in view of community-acquired VRE and to demonstrate the possible role of the food chain in the spread of VRE? The same vigilance given to methicillin-resistant *Staphylococcus aureus* should be applied to VRE as well and precautionary measures may be necessary before a real epidemic ensues and endemicity becomes the norm as had happened in the United States. What types of isolation policies are needed for different sources of VRE?—stool carriage only, discharging wounds, ICU patients with multiple lines. Molecular techniques are needed quickly to confirm the genotypicity of VRE isolates obtained thus far and to investigate their DNA-relatedness in order to understand the epidemiology and transmission of VRE in Singapore. How many of the HICPAC recommendations are applicable to hospitals in Singapore and what modifications are necessary for some of them?

There is a need for microbiologists and clinicians to detect, prevent and control VRE in Singapore using current information while it is still in its infantile stage. Herein lies the beauty of preparedness in the endless saga of man versus microbes.

REFERENCES

1. Centers for Disease Control and Prevention. Nosocomial enterococci resistant to vancomycin—United States, 1989–1993. *MMWR Morb Mortal Wkly Rep* 1993; 42:597–9.
2. Landman D, Mobaraki N K, Quale J M. Novel antibiotic regimens against

- Enterococcus faecium* resistant to ampicillin, vancomycin and gentamicin. Antimicrob Agents Chemother 1993; 37:1904-8.
3. Hakenbeck R. Resistance to glycopeptide antibiotics. In: Ghuysen J M, Hakenbeck R, editors. Bacterial Cell Wall. New York: Elsevier, 1994:535-45.
 4. Leclercq 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.
 5. Uttley A H C, Collins C H, Naidoo J, George R C. Vancomycin-resistant enterococci. Lancet 1988; 1:57-8.
 6. Jones R N, Sader H S, Erwin M E, Anderson S C. Prevalence data from 97 medical center surveillance study in the United States. Diagn Microbiol Infect Dis 1995; 21:85-93.
 7. Paul S M, Gursky E A. A statewide surveillance for antibiotic-resistant bacteria—the New Jersey Department of Health. Infect Control Hosp Epidemiol 1996; 17:345.
 8. Frieden T R, Munsiff S S, Low D E, Willey B M, Williams G, Faur Y, et al. Emergence of vancomycin-resistant enterococci in New York City. Lancet 1993; 342:616-7.
 9. Nosocomial enterococci resistant to vancomycin—United States, 1989-1993. MMWR Morb Mortal Wkly Rep 1993; 42:597-9.
 10. Ang S W, Seah C S, Lee S T. Vancomycin-resistant *Enterococcus* in the Singapore National Burns Centre: a case report. Ann Acad Med Singapore 1996; 25:270-2.
 11. Chiew Y F, Ling M L. Two cases of vancomycin-resistant enterococci from Singapore. J Infect 1997 (In press).
 12. Oon L L E, Chiew Y F. Stool carriage of vancomycin-resistant enterococci in a Singapore teaching hospital. Proceedings and abstracts of the Joint Scientific Meeting of Royal College of Pathologists of Australasia; 1997 October 6-9; Singapore. Singapore: Singapore Society of Pathology and Malaysian Society of Pathologists, 1997.
 13. Bonten M J, Hayden M K, Nathan C, van Voorhis J, Matushek M, Slaughter S, et al. Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. Lancet 1996; 348:1615-9.
 14. Noskin G A, Stosor V, Cooper I, Peterson L R. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. Infect Control Hosp Epidemiol 1995; 16:577-81.
 15. Chiew Y F, Hall L M C. Comparison of three methods for the molecular typing of Singapore isolates of enterococci with high-level aminoglycoside resistances. J Hosp Infect (In press).
 16. Joshi N, Milfred D, Caputo G. Vancomycin-resistant enterococci: a review. Infect Dis Clin Prac 1996; 5:528-37.
 17. Livornese L L, Dias S, Samei C, Romanovski B, Taylor S, May P, et al. Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic thermometers. Ann Intern Med 1992; 117:112-6.
 18. Karanfil L V, Murphy M, Josephson A, Gaynes R, Mandel L, Hill B C, et al. A cluster of vancomycin-resistant *Enterococcus faecium* in an intensive care unit. Infect Control Hosp Epidemiol 1992; 13:195-200.
 19. Montecalvo M A, Horowitz H, Gedris C, Carbonaro C, Tenover F C, Issah A, et al. Outbreak of vancomycin-, ampicillin-, and aminoglycoside-resistant *Enterococcus faecium* bacteremia in an adult oncology unit. Antimicrob Agents Chemother 1994; 38:1363-7.
 20. Spera R V, Farber B F. Multidrug-resistant *Enterococcus faecium*: an untreatable nosocomial pathogen. Drugs 1994; 48:678-88.
 21. Kaplan A H, Gilligan P H, Facklam R R. Recovery of resistant enterococci during vancomycin prophylaxis. J Clin Microbiol 1988; 26:1216-8.
 22. Emori T G, Gaynes R P. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin Microbiol Rev 1993; 6:426-42.
 23. Bingen E H, Denamur E, Lambert-Zechovsky N J, Elion J. Evidence for the genetic unrelatedness of nosocomial vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. J Clin Microbiol 1991; 29:1888-92.
 24. Witte W, Klare I. Glycopeptide-resistant *Enterococcus faecium* outside hospitals: a commentary. Microbial Drug Resistance 1995; 1:259-63.
 25. Klare I, Heier H, Claus H, Witte W. Environmental strains of *Enterococcus faecium* with inducible high level resistance to glycopeptides. FEMS Microbiol Lett 1993; 106:23-90.
 26. Torres C, Reguera J A, Sanmartin M J, Perez-Diaz J C, Baquero F. vanA-mediated vancomycin-resistant *Enterococcus spp.* in sewage. J Antimicrob Chemother 1994; 33:553-61.
 27. Reynolds P E. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur J Clin Microbiol Infect Dis 1989; 8:943-50.
 28. Dutka-Malen S, Leclercq R, Coutant V, Duval J, Courvalin P. Phenotypic and genotypic heterogeneity of glycopeptide resistance determinants in gram-positive bacteria. Antimicrob Agents Chemother 1990; 34:1875-9.
 29. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J Bacteriol 1993; 175:117-27.
 30. Evers S, Quintiliani Jr R, Courvalin P. Genetics of glycopeptide resistance in enterococci. Microbial Drug Resistance 1996; 2:219-23.
 31. Meziane-Cherif D, Badet-Denisot M, Evers S, Courvalin P, Badet B. Purification and characterization of the VanB ligase associated with type B vancomycin resistance in *Enterococcus faecalis* V583. FEMS Microbiol Lett 1994; 354:140-2.
 32. Dutka-Malen S, Molinas C, Arthur M, Courvalin P. Sequence of the vanC gene of *Enterococcus gallinarum* BM4147 encoding a D-alanine:D-alanine ligase related protein necessary for vancomycin resistance. Gene 1992; 112:53-8.
 33. Swenson J M, Ferraro M J, Sahm D F, Charache P, Tenover F C. New vancomycin diffusion breakpoints for enterococci. J Clin Microbiol 1992; 30:2525-8.
 34. Jordens J Z, Bates J, Griffiths D T. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J Antimicrobial Chemother 1994; 34:515-28.
 35. Swenson J M, Clark N C, Ferraro M J, Sahm D F, Doern G, Pfaller M A, et al. Development of a standardized screening method for detection of vancomycin-resistant enterococci. J Clin Microbiol 1994; 32:1700-4.
 36. Edberg S C, Hardalo C J, Kontnick C, Campbell S. Rapid detection of vancomycin-resistant enterococci. J Clin Microbiol 1994; 32:2182-4.
 37. van-Horn K G, Gedris C A, Rodney K M. Selective isolation of vancomycin-resistant enterococci. J Clin Microbiol 1996; 34:924-7.
 38. Rao G G, Ghanekar K, Ojo F. Selective medium for screening for vancomycin-resistant enterococci in faeces. Eur J Clin Microbiol Infect Dis 1996; 15:175-7.
 39. Cartwright C I, Stock F, Fahle G A, Gill V J. Comparison of pigment production and motility tests with PCR for reliable identification of intrinsically vancomycin-resistant enterococci. J Clin Microbiol 1995; 33:1931-3.
 40. van-Horn K G, Gedris C A, Rodney K M, Mitchell J B. Evaluation of commercial vancomycin agar screen plates for detection of vancomycin-resistant enterococci. J Clin Microbiol 1996; 34:2042-4.
 41. Willey B M, Kreiswirth B N, Simor A E, Faur Y, Patel M, Williams G, et al. Identification and characterization of multiple species of vancomycin-resistant enterococci, including an evaluation of Vitek software version 7.1. J Clin Microbiol 1993; 31:2777-9.
 42. Fontana R, Ligozzi M, Pittaluga F, Satta G. Intrinsic penicillin resistance in enterococci. Microbial Drug Resistance 1996; 2:209-13.
 43. Moellering R C Jr, Wennersten C B G, Weinberg A N. Studies on antibiotic synergism against enterococci: II. Effect of various antibiotics on the uptake of ¹⁴C-labeled streptomycin by enterococci. J Clin Invest 1971; 50:2580-4.
 44. Chiew Y F. *Enterococcus*: a much neglected bug. NUH CME Rev 1993; 3:8-12.
 45. Low D E. Quinupristin/dalfopristin: spectrum of activity, pharmacokinetics, and initial clinical experience. Microbial Drug Resistance 1995; 1:223-34.
 46. Lynn W A, Clutterbuck E, Want S, Markides V, Lacey S, Rogers T R, et al. Treatment of CAPD-peritonitis due to glycopeptide-resistant *Enterococcus faecium* with quinupristin/dalfopristin. Lancet 1994; 344:1025-6.
 47. Nachamkin S A, Verma R, Egnor M. Vancomycin-resistant *Enterococcus faecium* shunt infection in an infant: An antibiotic cure. Microbial Drug Resistance 1995; 1:95-6.
 48. Sahgal V S, Urban C, Mariano N, Weinbaum F, Turner J, Rahal J J, et al. Quinupristin/dalfopristin (RP 59500) therapy for vancomycin-resistant *Enterococcus faecium* aortic graft infection: case report. Microbial Drug

- Resistance 1995; 1:245-7.
 49. Jones R N, Johnson D M, Erwin M E. In vitro antimicrobial activities and spectra of U-1000592 and U-1000766, two novel fluorinated oxazolidinones. *Antimicrob Agents Chemother* 1996; 40:720-6.
 50. Urban C, Mariano N, Mosinka-Snipas K, Wade C, Chahrour T, Rahal J J. Comparative in-vitro activity of SCH 27899, a novel everninomicin, and vancomycin. *J Antimicrob Chemother* 1996; 37:361-4.
 51. Biavasco F, Lupidi R, Varaldo P E. *In vitro* activities of three semisynthetic amide derivatives of teicoplanin, MDL 62208, MDL 62211, and MDL 62873. *Antimicrob Agents Chemother* 1992; 36:331-8.
 52. Peetermans W E, Sebens F W, Guiot H F. Vancomycin-resistant *Enterococcus faecalis* in a bone-marrow transplant recipient. *Scand J Infect Dis* 1991; 23:105-9.
 53. Lai K K. Treatment of vancomycin-resistant *Enterococcus faecium* infections. *Arch Intern Med* 1996; 156:2579-84.
 54. Recommendations for preventing the spread of vancomycin resistance. Hospital Infection Control Practices Advisory Committee (HICPAC). *Infect Control Hosp Epidemiol* 1995; 16:105-13.
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