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INFORMASI LABORATORIUM MEDIK TERBARU
INTRODUCTION

Enterococci are normal commensals in colon of people as well as animals. They are Gram-positive cocci, Enterococcus faecalis and Enterococcus faecium are the two major species. Other species are E. avium, E. casseliflavus, E. durans, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. raffinosus, E. solitarius and E. pseudoavium.1,2 Enterococcus infections can occur in the urinary tract, blood and wounds, including surgical wounds.3 Over the last two decades, enterococci, formerly viewed as organisms of minimal clinical impact, have emerged as an important hospital-acquired pathogen in immunosuppressed patients as well as these in intensive care units.4

Vancomycin-resistant enterococci (VRE) have become one of the major threats to public health in many parts of the world. VRE represent a reservoir of glycopeptide resistance in hospital patients, especially in high-risk wards.5,6 VRE is thought to be passed to people through contact with animals or by consuming animal meat. Once they consumed the bacteria stay dormant in the person's gut until they become into contact with an antibiotic. At that point the VRE can spread to the rest of the body. Since many patients in hospitals are treated with some kind of antibiotic therapy during the course of treatment, VRE infections often occur in hospitals.3

Currently, there are no known effective antimicrobial agents to treat infections caused by VRE (prevention and early detection the best approaches to control it) and VRE can remain viable in the environment for an extended time period, and therefore pose a problem for infection control in hospitals and nursing homes. In addition, these enterococci have been detected as part of the enteric flora in non-symptomatic patients. These colonized patients serve as potential sources to transfer these organism to other patients and medical personnel.7

ENTEROCOCCAL INFECTION

Enterococci do not rise in unique clinical syndromes. Enterococci may colonize open wounds, urine, and occasionally sputum without causing clinical symptoms. The only commonly caused infections are urinary tract, intra-abdominal and soft tissue infections and bacteremia. Skin and soft tissue infections are rarely occur due enterococci, although its frequently colonized wounds, pressure ulcers and other skin lesions.4
Most clinical isolates are either *E. faecalis* or *E. faecium*. *E. faecalis* accounts for 80 to 90% of enterococcal infections from all sources, with *E. faecium* responsible for the rest of majority. That matter typically can cause urinary tract infections (UTIs), bloodstream infections, endocarditis and intra-abdominal infections. Nosomial bacteremias are increasing in numbers. Portals entry for enterococcal bacteremia include the urinary tract, intra-abdominal (or pelvic) sepsis, wounds (especially thermal burns, decubitus ulcers, or diabetic foot infections), intravenous or intra-arterial catheters, or cholangitis.

More recently, VRE has emerged as a significant pathogen in the immunocompromised population, particularly in patients who have received multiple courses of antibiotics or have been hospitalized for a prolonged period of time. Bloodstream infection with enterococci can be associated with serious morbidity. Septic shock may occur in up to 62% of patients with enterococcus bloodstream infection and the associated crude mortality has been reported to be 48%.

**VANCOMYCIN RESISTANT ENTEROCOCCI**

Glycopeptides Resistance

Vancomycin and teicoplanin are the two main glycopeptide antibiotics in clinical use. They are active only against Gram-positive species and have a similar mechanism of action. Glycopeptides inhibit bacterial cell wall synthesis. They are effective at inhibiting the growth of enterococci including *E. faecium*. However, the killing (bactericidal) effect of glycopeptides against enterococci when used as single agents is limited.

![Figure 1. Schematic diagram of mechanism resistance against vancomycin](image)

Vancomycin-susceptible enterococci synthesize cell-wall precursors ending in D-Ala-D-Ala, which, after translocation from the cytoplasm to the cell surface, bind vancomycin with high affinity; once bound, these precursors cannot participate in cell-wall synthesis (Figure 1). Vancomycin-resistant enterococci, in the presence of an inducer like vancomycin, generate precursors with different termini (D-Ala-D-Lac, D-Ala, or D-Ala-D-Ser), which have low affinity for vancomycin and thus can continue, in large part, to be used to synthesize cell wall. Ala denotes alanyl or alanine, and X lactate for VanA, VanB, and VanD types of resistance and serine for VanC and VanE types.

There are six types of glycopeptide resistance reported in enterococci (VanA, VanB, VanC, VanD, VanE and VanG). VanA, which is the most studied mechanism of resistance, is encoded as a cluster of seven genes on a mobile genetic element (Tn1546). The genetic element confers the ability to synthesize the bacterial cell wall in a way that bypasses the inhibitory effect of vancomycin. The distinction of interest between VanA and VanB type resistance is that the VanA mechanism is associated with resistance to vancomycin and teicoplanin; by contrast, VanB isolates are sensitive (at least on laboratory testing) to teicoplanin but resistant to vancomycin. Despite the in vitro sensitivity of enterococci with a VanB mutation to teicoplanin, therapy of such strains with teicoplanin has often been unsuccessful and the emergence of resistance during treatment has been described (Table 1). Enterococci are known acquire antibiotic resistance with relative ease and able to spread these matter genes to other species. *Enterococcus faecalis* has been reported to transfer plasmids harbouring antibiotic-resistance traits to other enterococci and to *Listeria monocytogenes* in water treatment plants. *Enterococcus faecium* conjugative transposons can be transferred from animal bacteria to human ones. Such conjugative transposons can also transfer vancomycin resistance to *Staphylococcus aureus*, streptococci and lactobacilli.

Although *E. faecalis* is more common in human infections, vancomycin resistance is more frequently observed in *E. faecium* isolates. Most vancomycin-resistant *E. faecium* (VREF) strains isolated in Korea showed the VanA phenotype, and VREF isolates with the VanB phenotype have been reported in Korea since 1997. Generally, the vanA gene cluster confers the vanA phenotype and vanB gene cluster is associated with the VanB phenotype. Recently, however, VRE strains with the vanA gene and VanB phenotype. However, VRE strains with the vanA gene and VanB phenotype have been found in Japan, Taiwan, and Korea. VREF is an important concern not only because its infection is difficult to treat in clinical practice but also because its clones can spread within hospitals as well as between regions or countries.
Motile enterococcal species, *E. gallinarum* and *E. casseliflavus*, usually carry the vanC1 or vanC2 gene, respectively, and exhibit low-level intrinsic resistance to vancomycin. These VRE species are found as normal stool flora and are not usually considered clinically significant even though sporadic bloodstream infections have been detected in severely immunocompromised patients. Together these species are responsible for about 1–2% of all enterococcal infections in humans.

**Epidemiology of VRE**

The first case reports of VRE due to VanA type resistance in strains of *E. faecium* derivat patients from England and France in 1986. The first VanB isolate was reported in Missouri, US, in 1987. Since then, rates of VRE have increased and studies have shown a rectal carriage rate of 5 up to 10% in the Netherland and 11.8% in France among the general population. When looking at high-risk populations (haematology, oncology, ICU etc), European countries have high carriage rates of VRE, with 16.3% in Berlin, 14% in Belgium and 18% in Copenhagen. In the US in 2000, 25.9% of enterococci isolated from blood were resistant to vancomycin, having been almost negligible in 1989. In Ireland the rate of VRE causing bloodstream infections has also been increasing. In the last quarter of 2006, 41.3% of *E. faecium* bloodstream infections were vancomycin resistant and this has been increasing since 2002.

Furtado et al. (2005) reported that in Brazil there was a progressive increase in the vancomycin resistance in the clinical cultures that were positive for *Enterococcus spp.*, over the three years of the study. In 2000, 9.5% of the samples were vancomycin-resistant, and this increased to 14.7% in 2001 and 15.8% in 2002.
The spread of VRE has been multifactorial, related in part to excessive antibiotic use among humans and animals, but also to acquisition of VRE in the healthcare setting related to the ease with which this organism spreads from person to person and inadequate infection control practice. Avoparcin, a glycopeptide antibiotic similar to vancomycin, which is used in animal husbandry, is thought to have been a factor in the high rates of VRE colonisation among poultry. Due to its limited potency against staphylococci, the use of avoparcin may also represent a risk for the development of glycopeptide resistant staphylococci. When animals consume avoparcin-supplemented feeds, vancomycin-susceptible strains may undergo selection for resistance to the antibiotic. VRE were isolated from meat and human faecal flora in countries with heavy use of avoparcin. VRE have been isolated from pets.

Most VRE infections occur in hospitals and involve infection of the urinary tract, blood stream or wounds. Those at higher risk of becoming infected with VRE include: Persons who have previously been treated with vancomycin and combinations of other antibiotics like penicillin and gentamicin. Persons who are hospitalized, especially when they receive antibiotic treatment for long periods of time. Persons with weakened immune systems such as patients in Intensive Care Units, in cancer or transplant wards, or who are infected with HIV. Persons who have undergone surgical procedures such as abdominal or chest surgery and Persons with medical devices that stay in for some time, such as urinary catheters or central intravenous catheters.

**LABORATORY TEST**

Experience has shown that many patients, probably most, who acquire VRE carry it in their gastrointestinal tract for periods of time without suffering any adverse health effects. This is referred to as VRE colonisation. Patients can be screened for colonization with VRE by culturing specimens (typically rectal swabs) on culture media that favour the growth of enterococci and that have added vancomycin. Bacteria resembling enterococci which are cultured on these vancomycin-containing agar plates are then tested further to verify if they are VRE. Screening for VRE is performed quite selectively in many healthcare settings with an emphasis on groups of patients most vulnerable to infection with VRE, such as immunocompromised patients on chemotherapy.

Resistance to vancomycin can be detected by the E-test (AB Biodisk, Solna, Sweden). An inoculum with turbidity equivalent to that of a 0.5 Mc Farland standard and Mueller-Hinton agar were used. Plates were read after incubation at 37° C for 24 h, and the minimum inhibitory concentrations (MICs) obtained by the E-test were rounded to the nearest higher doubling dilution.

VRE at the Nebraska Public Health Laboratory (NPHL) are generally detected by routine "Aerobic Culture" of a normally sterile body site, by a "VRE Culture Screen", or as an incidental finding during the culture of stool for "Enteric Pathogens". Gram positive cocci with atypical macroscopic appearance, which are catalase-negative and spot pyrrolidonyl arylamidase-(PYR) positive are suspected Enterococcus species. Also, growth of an isolate with these characteristics on an enteric pathogen screen culture to detect Campylobacter in stool, should be considered suspicious for the presence of VRE. This medium supports the growth of enterococci and contains vancomycin in a concentration adequate to screen for resistance.

Suspected enterococcal isolates considered clinically significant or isolates which grow on CVA medium are subsequently tested by biochemicals for identification and by tests for susceptibility to, high-levels of gentamicin, high-levels of streptomycin, and ampicillin. Additionally, an agar dilution test-containing vancomycin is also inoculated as an initial screen for vancomycin resistance. Isolates identified as Enterococcus species which grow in the presence of vancomycin on the agar dilution plate are subsequently confirmation tested. This includes vancomycin and teicoplanin disk diffusion and motility tests to screen for the low-level vancomycin resistant motile enterococci (vanC strains). Isolates confirmed as nonmotile and by the disk diffusion as resistant to vancomycin, are identified as VRE.

Several PCR-based methods for the specific detection of enterococci have been reported. PCR can be used to discriminate between different kinds of genes encoding resistance to glycopeptides. In this instance, primers that specifically hybridize to different conserved DNA sequences in the different resistant genotypes are used to amplify particular gene fragments that allow different resistance types to be distinguished. One such method is based on targeting the ddl gene, permitting the specific detection of E. faecalis and E. faecium. Recently, a Multiplex PCR assay that allows simultaneous detection of glycopeptides resistance genes (vanA, vanB, vanC-1) and identification at the species level, of clinically relevant enterococci (E. faecium, E. faecalis, E. gallinarum, E. casseliflavus) has been developed. PCR-RFLP assay can be performed directly with isolated colonies of Enterococcus spp. to detect and discriminate vanA, vanB, vanC-1 and vanC-2/3 genes. The recent cloning and characterization of the
vanD and vanE genes will permit the development of PCR assays for the specific detection of these new types of glycopeptide resistance. Subsequent DNA sequencing of the PCR fragments gives information on the variability of glycopeptide resistance genes within each type of resistance class.20

**MANAGEMENT**

The most important decision when managing a patient with VRE isolated from a specimen is the clinical judgement as to whether the patient has VRE infection or VRE colonization. Given that many patients in the community have VRE colonization, it should be apparent that isolation of VRE by the laboratory should not automatically result in antibiotic prescription. The decision to treat is based on the clinical condition of the patient together with the site from which the VRE was isolated, other laboratory data and imaging studies if appropriate.1 Patients infected or colonized with VRE may be cared for to any patient care setting with minimal risk of transmission with other patients provided appropriate infection control measures are taken.2

Where therapy is required, there are some therapeutic options for VRE, such as quinupristin-dalfopristin, teicoplanin, linezolid, daptomycin and tigecycline. In the late 1999, quinupristin-dalfopristin became the first antimicrobial agent available for the treatment of vancomycin-resistant *E. faecium* infection. Although *E. faecium* is susceptible to quinupristin-dalfopristin, most *E. faecalis* isolates and many other non-*E. faecium* species are intrinsically resistant to this antimicrobial agent. Quinupristin-dalfopristin, a streptogramin, targets the bacterial 50S ribosome, thereby inhibiting protein synthesis. Although uncommon, resistance can develop through modification of the target binding site, enzymatic inactivation, and/or efflux.11

Most VRE isolates express the vanA phenotype and are resistant to teicoplanin. However, there remain some areas where vanB isolates (which are susceptible to teicoplanin) occur with equal frequency. Unfortunately, vanB enterococci have demonstrated the potential to readily develop teicoplanin resistance *in vitro* and *in vivo*, a finding that seriously limits the usefulness of this agent.8

The oxazolidinones (linezolid) exhibit a unique mechanism of protein synthesis inhibition and display bacteriostatic activity against Gram positive pathogens, including methicillin-resistant *Staphylococcus aureus*, VRE (both *E. faecalis* and *E. faecium*), and drug-resistant *Streptococcus pneumoniae*. It can be given orally or intravenously and it has very good bioavailability. Linezolid is licensed for pneumonia and complicated skin and soft-tissue infections caused by Gram-positive bacteria. It achieves good penetration into osteoarticular tissues as well as into intrapulmonary tissues.1,4

Daptomycin (Cubicin) became available in 2003 for the treatment of VRE infection. It is from a novel class of antibiotics called cyclic lipopeptides and has activity against VRE *in vitro*. Its mechanism of action is by disrupting the bacterial cell membrane leading to cell death with negligible cell lysis. It is licensed for the treatment of complicated skin and soft tissue infection in adults caused by susceptible Gram-positive pathogens.1,11

Tigecycline is a glyvcycline antibiotic, which is similar to the tetracycline class of antibiotics. It is indicated for the treatment of complicated skin and soft tissue infections, as well as complicated intra-abdominal infections. It is only given intravenously and there is no oral formulation. There is limited experience with the use of this agent for the treatment of VRE infection.1,11

Ramoplanin is a member of the new glycolipodepsipeptide class of antibiotics. It is highly active *in vitro* against all enterococcal species, and against VRE. A Phase III study is currently investigating its use in the prevention of VRE bacteraemia in VRE-colonized patients in the USA. Ramoplanin could potentially play a part in a new infection control strategy for VRE.21

**INFECTION CONTROL**

Persons infected or colonized with VRE are more likely transmit the organism. Transmission depends primarily on which body site(s) harbored the bacteria, whether the body fluids are excreted and how frequently health care providers touch these body sites. Body fluids which are excreted create a risk for transmission of the organism and, possibly, contamination of the environment. Reinforce employee education about basic infection control measures, especially hand washing between patient contacts. Patient care equipment should be dedicated for patients identified with VRE (i.e., blood pressure cuffs, thermometer, stethoscopes), otherwise, shared equipment should be appropriately cleaned and disinfected between patient use.2

In another report from an intensive care unit, VRE acquisition was similar whether gowns with gloves or gloves alone were used for direct patient care. There are three different intervention approaches to contain VRE on these different wards. These included enhanced environmental decontamination on one unit, intensive continuing re-education on infection control policies and precautions on a second unit, and replacement of disposable oral and rectal thermometers by tympanic thermometers for all temperatures on a third unit.22
Guidelines on reducing the spread of VRE have been published by the Centers for Disease Control and Prevention. They emphasize the need for prudent use of vancomycin and continuing education of all hospital staff regarding VRE. In addition, laboratories are encouraged to screen and report cases of VRE. Finally, the guidelines focus on the prevention and control of nosocomial transmission of VRE. It is important that the patient is isolated in a single room. Hand hygiene should be practiced on entering and leaving the room and everybody should wear a plastic apron when entering the room. Non-critical medical equipment, such as thermometers and blood pressure measuring devices, should be used only on the patient with VRE and not used on other patients.1

DISCUSSION

Enterococci are found in the intestinal tract of virtually all humans where they help form part of the normal human flora. Because of their ability to withstand harsh conditions, they can survive in soil, food, water and in a wide variety of animals as well. Of the many different species of enterococci, two account for the vast majority of human infections: E. faecalis (the most common) and E. faecium. The spectrum of illness caused by these organisms ranges from simple uncomplicated urinary tract infections to serious, life-threatening infection of the heart valves known as endocarditis. Their role in other infections such as intra-abdominal or pelvic infections is somewhat controversial given the fact that in these situations they are usually found mixed with other bacteria and patients often improve clinically when treated with antibiotics that are not active against enterococci. Culture techniques are used to isolate VRE in order to diagnose infection, assess antimicrobial susceptibility and identify clonality in the case of nosocomial outbreaks.

Despite variability in clinical presentations and outcome, VRE infections represent important clinical and public health problems: VRE are an important cause of nosocomial infections emphasizing that efforts to prevent infections are essential in health care settings. Resistance can be transferred between organisms, increasing the potential emergence of vancomycin-resistance in clinical isolates of Staphylococcus aureus and Staphylococcus epidermidis. Already, methicillin-resistant S. aureus (MRSA) infections are widespread in many hospitals in the United States, and vancomycin is the drug used to treat these infections. Persons with VRE infection or colonized increase complexity of patient transfer particularly between the acute-care settings and the long-term care settings.

Portals of entry for VRE include the urinary tract, intra-abdominal (e.g. the gastrointestinal tract including the biliary tree) or pelvic sources, wounds (e.g. surgical wounds, decubitus ulcers) and intravascular catheters. Isolation of VRE from the urine alone is of limited clinical significance; most urine cultures yielding VRE do not represent true infection but rather asymptomatic bacteriuria.

The most likely modes of transmission from patient to patient are either by direct contact through transient carriage of VRE on the hands of personnel, or indirectly by contaminated environmental surfaces and patient care equipment. The emergence of vancomycin resistance in enterococci suggests that preassembled operons, or parts thereof, were transferred to enterococci, before or at that time, and selected for under the pressure of dramatically increased glycopeptide use in clinical practice (e.g. vancomycin) and/or in animal husbandry (e.g. avoparcin).

VRE colonization, predominantly of the gastrointestinal tract, precedes infection. VRE intestinal colonization does not result in symptoms, may last for long periods and serves as a reservoir for transmission of VRE to other patients. VRE colonization is an important factor leading to nosocomial dissemination of the organism. Also, VRE colonization independently increases a infections patient’s risk of developing infections, such as bloodstream.

Linezolid is the antimicrobial used most commonly to treat infection with VRE. Other antimicrobials such as quinupristin-dalfopristin, daptomycin, tigecycline, and nitrofurantoin are also prescribed. Currently, no accepted treatment for colonization has been determined. Knowledge about VRE is important for all health care professionals. With their increasing prevalence, capacity for prolonged survival in the environment, ability to overcome infection-control procedures, and capability of transferring vancomycin resistance to S. aureus, VRE represent an important infectious disease threat.

Strict adherence to appropriate infection control guidelines for the prevention of VRE transmission in hospitals and tracking of VRE colonization through active surveillance in high-risk units are recommended. Avoidance of glycopeptide use in animal husbandry and prudent use of antimicrobial agents in human medicine are also recommended. Certain antimicrobial agents have been associated with an increased risk of acquisition of colonization with VRE. In humans with established VRE colonization, antimicrobial agents with anti-anaerobic activity, as compared with those without, have been associated with high-density colonization with VRE. There is a continued need for the development of new

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antimicrobial agents for treating VRE infection, as well as a regimen that would eradicate VRE colonization (without selection of further antimicrobial resistance), and potentially a role for a regimen for suppressing VRE colonization during periods of high risk for enterococcal infection.

CONCLUSION

Vancomycin-resistant enterococci (VRE) have become one of the major threats to public health in many parts of the world. VRE is thought to be passed to people through contact with animals or by eating animal meat. Since many patients in hospitals are put on some kind of antibiotic therapy during the course of their treatment, VRE infections often occur in hospitals. Vancomycin resistance is more frequently observed in E. faecium isolates.

While there are some therapeutic options available that can treat VRE in vitro, there are no licensed options for VRE bloodstream infection. This is a particular cause for concern because of the frequency of VRE bloodstream infection sepsis that is now occurring worldwide. This leaves the most vulnerable patient populations (ICU, haemodialysis, haematology/oncology) with a limited range of antibiotics to manage VRE bloodstream infection. It is important that infection control policies and antibiotic guidelines are implemented to control the spread of VRE and other multi-drug resistant organisms.

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