Vancomycin Resistant *Enterococcus* (VRE): Prevalence and Characteristics in a Tertiary Hospital In Malaysia

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ABSTRACT

Introduction: Vancomycin-resistant *Enterococcus* (VRE) are undoubtedly less virulent as compared to other common pathogenic bacteria such as *Staphylococcus aureus.* However the presence of VRE is a matter of concern, as VRE infections are associated with high mortality, particularly in immunocompromised patients. The resistant gene is transferable to Methicillin-Resistant *Staphylococcus aureus* (MRSA). **Materials and Methods**: VRE cases from clinical samples in a tertiary government hospital were identified retrospectively over a period of 12 months. VRE genotype was confirmed by molecular method. Patients' clinical data were obtained from the hospital information system. **Results**: 2.88% (n=7/243) of all *Enterococcal* spp isolated from clinical samples were resistant to vancomycin (VRE). All were from haematological patients, six were diagnosed with neutropaenic sepsis. All VRE were isolated within eight to 37 days after chemotherapy. Six out of seven cases where VRE were isolated had received carbapenem in their therapy. There were four samples from central venous catheter, one from peripheral blood, one from pus and one from urine sample. Only three patients were treated with Linezolid. However, all patients recovered well. All isolates carry *vanA* gene with vancomycin MIC of >256 ug/mL. **Conclusions**: The prevalence of VRE in this current study is higher than an earlier study done in Malaysia. This finding showed that patients from haematological ward had higher risk of VRE colonization or infection. Periodic screening is necessary to monitor the prevalence of multidrug resistant organism in order to encourage healthcare workers towards practicing proper infection control measures.

Keywords: Prevalence, Vancomycin-resistant *Enterococcus*, microbial resistance, sepsis, haematology

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INTRODUCTION

Enterococci are normal inhabitants of the gastrointestinal tract of humans and animals.

The two most common species responsible for human infections are *Enterococcus faecalis* and *Enterococcus faecium.* Vancomycin-resistant *Enterococci* (VRE) was first reported in 1988 in Europe. [1] The first case of hospital acquired VRE in Malaysia was reported in 1996 from the University Malaya Medical Centre. [2] In 2006, Kuala Lumpur Hospital, one of the largest general hospitals in Malaysia reported its first case in a patient with chronic renal failure. [3]

We identified cases of VRE in a tertiary government hospital for haematological disorders. It is a 500-bedded hospital with a total of 12 general wards, and four haematology wards. In 2013 there was a spike in sporadic VRE cases in the center, with total of seven non-duplicated cases. The objectives of this study were to determine the prevalence of VRE and to observe the clinical and laboratory characteristics of VRE isolated from clinical samples. Although *Enterococci*, including VRE, are undoubtedly less virulent as compared to other common pathogenic bacteria such as *Staphylococcus aureus,* the presence of VRE is a matter of concern. This is because VRE infections are associated with high mortality, particularly in immunocompromised patients and the resistant gene is transferable to Methicillin-Resistant *Staphylococcus aureus* (MRSA).

MATERIALS AND METHODS

Samples: Laboratory culture results with *Enterococcal spp* isolates were identified retrospectively from over a period of a year. The results of antibiotic susceptibility test were also obtained. Isolates that were resistant to vancomycin were further identified. The specimens were obtained from in-patients, during their acute clinical presentation. The isolation and identification of VRE were performed using standard methods in the microbiology laboratory within the hospital. The initial antimicrobial susceptibility test was by disk diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) guidelines and breakpoints. [4] Vancomycin resistant isolates were later subjected to minimum inhibitory concentration (MIC) test by Etest ®. Suspected isolates were then sent to the Institute of Medical Research (IMR), Kuala Lumpur for further analyses. VRE were confirmed by detection of *van* gene by PCR. Teicoplanin susceptibility was determined by the disk diffusion method.

Clinical history: The patients' clinical data including history, clinical presentations and microbiologic investigation results were obtained from the 'Hospital Information System'.

RESULTS

There were 243 *Enterococcal* spp isolated from all clinical samples. Seven cases of VRE were recognised, which accounted for a prevalence of 2.88%. All of VRE patients were diagnosed with haematological disorders, six of them from haematological ward and one was from the Intensive Care Unit (Table 1). Majority of the samples were from blood, one from peripheral blood and four from catheters at the femoral and intra-jugular veins. The remaining two samples were from pus and urine. Six of the seven patients had neutropenic sepsis at the time of specimen collection.

All patients received multiple antibiotics prior to VRE isolation (Table 2). Cases 3

Table 1: Vancomycin Resistant Enterococci (VRE) cases and their clinical characteristics.

Case No	Age	Sex	Underlying disease & diagnosis	Specimen	Antibiotic treatment	Outcome
1	59	M	AML M4 (myelomonocytic) with neutropenic sepsis	Urine	Linezolid	Discharged well
2	69	M	AML M4 with gluteal cellulitis	Pus	-	Discharged well
3	50	M	Refractory DLBCL Stage IIIb with CNS infiltration. Neutropenic sepsis	Blood (IJC)	Linezolid	Discharged well
4	50	M	AML with neutropenic sepsis	Blood	-	Discharged well
5	42	M	Relapsed DLBCL with CNS infiltration Neutropenic sepsis	Blood (femoral)	-	Discharged well
6	26	M	DLBCL of mediastinum Neutropenic Sepsis	Blood (femoral)	-	Discharged well
7	67	M	AML Neutropenic Sepsis	Blood (femoral)	Linezolid and gentamicin	Discharged well

AML: acute myelocytic leukaemia, CNS: central nervous system DLBCL: diffuse large B cell lymphoma, IJC: intra-jugular catheter

and 7, with the intra-jugular and femoral catheters were treated with a one-week course of linezolid, while Cases 5 and 6, with the femoral catheters were not treated for VRE. The patient with VRE positive urine sample was also treated with 10 days linezolid, whilst the patient with positive pus swab was not treated. Case 4, whose sample was taken from peripheral blood was not treated with antibiotic. Despite the differences in the samplings and management, all patients recovered well. Moreover, repeat sampling on the subsequent patients' admissions showed no isolation of VRE.

All seven isolates were identified to be *Entrococcus faecium*. The VRE were confirmed by detection of *vanA* gene in all isolates. All isolates were highly resistant to vancomycin with MIC of >256 µg/mL. Apart from being resistant to vancomycin, these isolates were also resistant to ampicillin and penicillin. Out of the seven isolates, five were resistant to also teicoplanin. However, they were all susceptible to linezolid. There are variations in the susceptibility patterns to other antibiotics namely, high level gentamicin and tetracycline (Table 3).

DISCUSSION

The prevalence of VRE in clinical isolates of *Enterococci* is increasing throughout the world. It varies from less than 2% in Finland to as high as 33% in the USA. [5] In Malaysia, the prevalence of VRE in the largest general hospital was reported to 1%. [6] However, the prevalence of VRE in this present study is higher. All of the cases in this study where VRE was isolated had haematological disorders. This finding shows that patients from haematological wards had higher risk of VRE colonisation or infection.

Reports showed that prolonged hospitalisation and inappropriate use of antibiotics were the most important factors for VRE colonisation or infection. [6] In this present study,

Table 2: List of antibiotics used prior to VRE isolation.

Case	Anti-mirobials received prior to VRE isolation
1	Gentamicin, Meropenem, Vancomycin, Amphotericin, Caspofungin
2	Meropenem, Metronidazole
3	Imipenem, Amikacin, Tazosin, Vancomycin
4	Meropenem
5	Meropenem, Gentamicin, Tazosin, Linezolid
6	Cefepime, Amphotericin
7	Meropenem, Sulperazone

Table 3: Laboratory Characteristics of Vancomycin Resistant Enterococci (VRE).

Case	*Enterococcus* spp	Antibiotic susceptibility (disk diffusion)							Van MIC (µg/mL)	Gene
		Amp	Gen	Lz	Pen	Tet	Tei	Van		
1	*E. faecium*	R	R	S	R	R	R	R	>256	VanA
2	*E. faecium*	R	R	S	R	R	I	R	>256	VanA
3	*E. faecium*	R	S	S	R	R	R	R	>256	VanA
4	*E. faecium*	R	S	S	R	R	I	R	>256	VanA
5	*E. faecium*	R	S	S	R	R	R	R	>256	VanA
6	*E. faecium*	R	R	S	R	R	R	R	>256	VanA
7	*E. faecium*	R	S	S	R	S	R	R	>256	VanA

Amp: ampicillin, Gen: high level gentamicin, Lz: linezolid, Pen: penicillin, Tei: teicoplanin, Tet: tetracycline, Van: vancomycin
R: Resistant; S : Sensitive; I: Intermediate

nisation or infection. [6] In this present study, VREs were isolated about eight to 37 days after hospital admission. All of these patients had initial episodes of neutropenic sepsis and had been given multiple courses of antibiotics including anti-fungal and anti-viral. Carbapenem (imipenem or meropenem) was the most common class of antimicrobial that were given to the patients, with the exception of one patient who was given vancomycin. In an earlier study done in Melbourne, Australia showed that hospital stay of ≥7 days and meropenem use were significantly associated with VRE detection. [7] This was also supported by a study done in Brazil. [8] Meanwhile, association between the use of vancomycin and VRE colonisation were shown by some authors to be inconsistent. [8, 9]

At least seven types of VRE have been described (VanA, VanB, VanC, VanD, VanE, VanG and VanL), that are named based on their specific ligase genes. VanA and VanB phenotypes are characterized by high-level resistance to vancomycin (minimum inhibitory concentrations of 64–1000 g/mL), which is inducible in nature. VanA phenotype also showed high-level resistance to the other glycopeptide and teicoplanin, but VanB phenotype is susceptible to teicoplanin. In this study, we found five isolates with VanA phenotype (resistance to both vancomycin and teicoplanin). Phenotype VanA and VanB are common in the USA, but phenotype Van C appears to be more prevalent in Europe.

We identified all our vancomycin resistant *Enterococci* were to be *Enterococcus faecium,* carrying *vanA* gene. Earlier reports from Malaysia also found all VRE isolates to carry *vanA* gene. However, the gene was not solely found in *Enterococcus faecium* species. [3, 6] Remarkably, majority of VRE isolates from other Asian countries for example Singapore and Australia belong to *vanB* genotype. [10, 11]

All our VRE isolates showed high MICs for vancomycin (>256 µg/mL) and at least intermediate sensitivity towards teicoplanin. There were cases of teicoplanin susceptible *vanA* genotype in other parts of East Asia as was reported China, Japan and South Korea. [12] This isolate is known as VanB phenotype-*vanA* genotype strain. For this type of isolates, teicoplanin, which is a cheaper antimicrobial, may be used instead of linezolid in the treatment of VRE infection. Therefore, teicoplanin MIC must be established in all VRE isolates in

order to determine their phenotype and the choice of treatment.

We also found four isolates that were considered as colonisers, thus no anti-VRE treatment were given. The question now is 'When should we treat VRE?' Apart from blood culture isolates, it is impossible to differentiate infection from colonisation in the non-screening isolates. For patients colonised with VRE, approximately 4% developed VRE bloodstream infection. [13] Nonetheless, up to 38% VRE colonised neutropaenic patients developed VRE related infections. [14] Immunosuppression and bloodstream infection had also been shown as independent risk factors for death related to VRE. [13] This study emphasised the vulnerability of patients with haemotological disorders thus periodic screening and early intervention are deemed necessary. Screening high-risk patient has been shown to help in anticipating subsequent infection. According to Liou *et al.*, negative VRE screening result had high specificity and NPV (negative predictive value) for the development of subsequent VRE infection. Therefore, empiric treatment of VRE infection may be unnecessary in VRE-negative patients. [15]

In conclusion, the findings from this study may cultivate awareness among healthcare workers on the presence of highly resistant organisms in the hospital environment. Control of multidrug resistant organism as VRE mainly lie on stringent infection control practices. Healthcare workers particularly those handling high-risk patients have the most responsibilities in ensuring minimum cross-transmission and contamination to their patients.

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