Enterococci: Emerging Drug Resistant Bacteria In Hospital Acquired Infections At Hospital Kuala Lumpur, Malaysia

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Citation

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Abstract

Enterococci are the most common pathogens in hospital acquired infections. Some of them are resistant to Vancomycin(VRE) and some are susceptible to Vancomycin (VSE). The present study was carried out to identify enterococci from clinical cases and to illustrate their clinical features and drug resistance characteristics. Antibiotics susceptibility of the identified bacteria was determined by disk diffusion method and E-test. Drug resistance properties were evaluated against ampicillin, gentamicin, vancomycin, teicoplanin and linezolid. Relating to clinical features, 244 cases of enterococci infected patients were identified at hospital Kuala Lumpur (HKL) Malaysia based on clinical information from the hospital. Of the patients 21% had history of urinary tract infections, 17.2% end stage renal disease, 12.2% sepsis, 8.4% malignancy, 12.2% had head injury and neurological problems, 4.2% diabetes mellitus and other clinical manifestations .In case of Vancomycin resistant enterococci infection, the clinical features of the patients were: end stage renal failure 3/6 (50%) and others with diabetes mellitus, interstitial lung disease and nephrotic syndrome. The patients of the enterococci infections were more prevalent in nephrology-urology unit (39%) and medical wards (including ICU, 23%) The findings would serve as an alert to the clinicians of the emergence of infections by enterococci and encourage implementation of appropriate infection control measures in order to curb further rise in prevalence.

INTRODUCTION

Enterococci normally present in the human intestines and in the female genital tract and are often found in the environment. Recent National Nosocomial Infections Surveillance[1] reveals that these enterococci remain in the top 3 most common pathogens that cause nosocomial infections. These are: urinary tract infections, bloodstream infections, and wound infections in hospitalized patients. Enterococci infections typically occur in very ill debilitated patients who have been exposed to broad-spectrum antibiotics. According to NNIS[2] data from January 2003 through December 2003, more than 28% of enterococci isolates were found to be associated infections in incentive care unit (ICU) of 300 participating hospitals.

The acquisition of vancomycin resistance by enterococci has seriously affects treatment and infection control program which leaves clinicians treating VRE infections with limited therapeutic options (Fraser, 2010)

NNIS[2] reported that more than 25% of health care-associated enterococcal infections were associated with organisms resistant to vancomycin. In Malaysia 2006, a confirmed case of vancomycin resistant Enterococci isolated from blood culture of a young woman with chronic renal failure was first reported in Hospital Kuala Lumpur (HKL).[3] However, later on, no systematic study was undertaken to determine the prevalence of enterococci infections from the hospitalized patients in Malaysia. Therefore, the present study was undertaken to determine the prevalence of enterococci infections in hospitalized patients and determination of their clinical features and drug resistance nature.

MATERIALS AND METHODS

STUDY AREA

The study was carried out during May 2007 to April 2008 at Hospital Kuala Lumpur (HKL), Malaysia. The hospital consists of 3000 beds and 90 wards and provides services and acts as a reference centre for the hospitals of other states, in Malaysia.

STUDY POPULATION AND SAMPLE COLLECTION

A total number of 244 samples obtained from the patients of different infections during the period. The samples included blood, urine, pus, tissue, body fluids and swabs from wound and placenta. The samples were sent to the Microbiology

Laboratory, Department of Medical Microbiology and Immunology, Faculty of Medicine, University Kebangsaan Malaysia for identification and characterization.

INFORMATION ON CLINICAL DATA

Information on patient's profiles :age, diagnosis, risk factors such as duration of hospitalization (prolonged >2 weeks), catheter use, usage of antibiotics like cephalosporin, carbapenem, vancomycin, recurrent admission and others such as malignancy, use of steroids were obtained from their medical records.

IDENTIFICATION OF ENTEROCOCCUS SPP

Enterococcal genus identification was based on Gram staining that showed gram positive cocci in pairs or short chains, growth and blackening of bile-esculin agar after overnight incubation , growth in the presence of 6.5% NaCl, absence of catalase (catalase negativity) and presence of pyrrolidonyl arylamidase (PYR). Streptococcal group antigens were also detected using group D antisera (Slidex Strepto-Kit; Bio Merieux, France). API 20Strep (BioMerieux, France) was used for species identification.

ANTIBIOTIC SUSCEPTIBILITY TEST

Antibiogram of the isolates was determined by disk diffusion method, testing for vancomycin (30µg), teicoplanin (30µg), ampicillin (10µg), high concentration of gentamicin (120µg) and linezolid (30µg). The tests were performed using the methodology recommended by the Clinical and Laboratory Standards Institute [4]

DISK DIFFUSION METHOD

Susceptibility test of enterococci to ampicillin, high level gentamicin, vancomycin, teicoplanin and linezolid was determined by a Kirby-Bauer disk diffusion method as per CLSI criteria [4]

E-TEST OF VANCOMYCIN

Minimum inhibitory concentration (MIC) for vancomycin was determined by E-test as per the procedure of CLSI[4] An isolate is considered susceptible to vancomycin if the MIC is ≤ 4µg/ml and resistant if MIC ≥ 32 µg/ml [4]

QUALITY CONTROL

Two QC strains Enterococcus faecalis ATCC 29212 and ATCC 51299 were used as sensitive and resistant controls, respectively. The MIC values of vancomycin for the control strains must be within the ranges provided by the Clinical and Laboratory Standard Institute[4] prior proceeding to test organisms.

MOLECULAR DETECTION OF VANOCOMYCIN RESISTANCE GENES

Vanocomycin-resistance genes were detected by PCR using specific primers as per the procedure of Boyd [5]et al. Briefly, PCR conditions consisted of a pre-denaturation step at 94°C for 5 min, followed by 30 cycles of 45 sec denaturation at 94°C, 45 sec annealing at 54°C and 45 sec extension at 72°C. A final extension step was performed at 72°C for 5 min. Amplified products were analyzed by electrophoresis on 1.5% agarose gel.

STATISTICAL ANALYSIS

All statistical analyses were carried out using SPSS version 12.0.

ETHICS COMMITTEE APPROVAL

The research work was approved by Ethics committee after finalization of the project proposal.

RESULTS

Enterococci of both vancomycin resistant and susceptible were isolated and identified from different clinical conditions (Table-1). It was observed that 21.5% enterococci were isolated from urinary tract infections, 18% from end stage renal failure, 12.2% from neurological problem, 12.3% from sepsis, 8.2% from malignancy, 7.4% from surgical problem, 4.1% from diabetes mellitus, 2.9% from motor vehicle accident with head injury, 2.5% from respiratory infections, 1.6% from neonatal infections, .8% from skin infections and .4% from nephritic syndrome

Figure 1

Table 1: The underlying clinical conditions for enterococci infections (VRE and VSE) at HKL, Malaysia

Clinical manifestations	Frequency		Percentage (%)
	VSE	VRE	
UTI	50		21.5
ESRF	41	3	18.0
Neurological problem	29		12.2
Sepsis (including urosepsis)	30		12.3
Malignancy	20		8.2
Others	20		8.2
Surgical problem	18		7.4
DM	10	1	4.1
MVA (with head injury)	7		2.9
Respiratory infections	5	1	2.5
Neonatal infection	4		1.6
Skin infections	2		0.8
Burn	2		0.8
Nephrotic syndrome	0	1	0.4
Total	238	6	100.0

ESRF=end stage renal failure
MVA= motorvehicle accident DM=Diabetes mellitus UTI= Urinary tract infection.

Enterococci identified from the patients of different wards and department at hospital KKL have been listed (Table-2). In the study 39% Enterococci were identified from the patients of nephrology ward, 23% from medical ward, 12% from surgical ward,13% from neurology ward,5% from pediatric, 3% from oncology/radiotherapy,2% from maternity, 1% from burn and 2% from out patient department(OPD)

Figure 2

Table 2: Distribution of vancomycin-resistant and vancomycin-susceptible Enterococci in different ward and departments at HKL, Malaysia

Wards/ Departments	*VRE N=6*	*VSE N=238*	Percentages (%)
Urology/Nephrology	4	92	39%
Medical (including ICU)	2	55	23%
Surgical	0	29	12%
Neurology	0	31	13%
Pediatric	0	11	5%
Radiotherapy/Oncology	0	8	3%
Maternity(O&G)	0	6	2%
Burn	0	2	1%
Others(Clinics, OPD)	0	4	2%

OPD=out patient department
ICU=intensive care unit, O & G= Obstetric and Gynaecology

Clinical features that were recorded for the infections of vancomycin resistant and susceptible patients were analyzed.

Figure 3

Table 3: Clinical features of vancomycin resistant and susceptible Enterococci infected patients at HKL, Malaysia

Patients	Total number of patients	Total (%)	No. of VRE	% of VRE	No. of VSE	% of VSE
Prolonged hospitalization	54	22.1	3	50	51	21.4
Catheter usage	70	28.7	1	16.7	69	29
Vancomycin treatment before	17	7	4	66.7	13	5.5
Cephalosporin treatment before	22	9	2	33.3	20	8.4
Carbapenem treatment before	21	8.8	0	0	21	8.8
Recurrent hospitalization	73	30	4	66.7	69	29
Malignancy	20	8.4	0	0	20	8.4

Out of all enterococci isolated 6 were found to be resistant to

vancomycin.

PCR results revealed that all the 6 vancomycin resistant enterococci possess Van A gene(In press)

DISCUSSION

Enterococci have both an intrinsic and acquired resistance to antibiotics,which make them important nosocomial pathogens.

Of all the enterococci infections analyzed (Table-3), out of 244 patients, 50 (21%) presented with urinary tract infections, 41 (17.2%) end stage renal disease, 29 (12.2%) neurological problems, 20 (8.4%) malignancy, 18 (7.4%) had surgical intervention and 10 (4.2%) had diabetes mellitus. Amongst the factors that described in this study to favor the infections, majority of them (29%) had recurrent hospitalization and catheter usage, followed by prolonged hospitalization, 21.4%. Although recurrent hospitalization and catheter usage were more frequent risks described in the enterococci infections, these risk factors were not statistically significant. Other risk factors like carbapenem and cephalosporin usage were also not statistically significant. Enterococci are rated as the second leading cause of urinary tract infections (UTI) and comprised about 10% of nosocomial UTI[6] and this study showed that UTI was the most common clinical manifestation of enterococci infections. Instrumentation like urinary catheter and urinary tract abnormality (urethral stricture, hydronephrosis)might have promoted infection with enterococci especially in elderly patients. In neurology units, most of the patients had limited mobility especially patients with cerebrovascular accidents and they usually used urinary and venous catheters that might have promoted enterococcal colonization and infections.

For patients with end stage renal disease, usually hospitalized frequently for dialysis. Some patients had dialysis via venous catheter (internal jugular catheter and/or femoral catheter) and this catheter was probably prone to be colonized with enterococci with the help of biofilm production. This group of patients had catheter related bloodstream infections and usually treated with intravenous antibiotics (cephalosporin, vancomycin, carbapenem) requiring prolonged hospitalization. The presence of an invasive device was identified previously as a strong clinical risk factor for VRE invasive infections[7]. It is unclear whether catheters served as the actual conduit through which VRE infection acquired, whether they were just markers of debilitation, prolonged hospitalization and severe co-morbidities. Patients with prolonged hospital stay were more susceptible to cross-transmission of pathogens and to a greater use of antimicrobials with selective pressure; therefore they had a greater chance of acquiring resistant pathogens. A previous study found that the duration of hospitalization was considerably longer to pick up infections of the VRE groups (57.7 days) compared to VSE group (29 days)[8]

Among the 6 VRE cases, 4 (66%) isolates were identified as E. faecium, 1 (17%) E. faecalis and 1 (17%) E. avium, respectively. Three patients (50%) had underlying end stage renal disease and the VRE was isolated from blood specimens. Two of them were admitted to intensive care units for severe sepsis, however, patients already expired when we informed the positive culture for VRE. Another patient from whom enterococci was isolated from the blood taken via the femoral catheter, also had coagulase negative staphylococcus species (CONS) isolated from the peripheral blood. He was treated for catheter related bloodstream infection before with intravenous vancomycin, however repeated blood culture revealed no growth. This may suggest that this patient had been colonized with VRE. He was discharged well after 3 weeks in the ward and subsequent culture after that was negative for VRE.

The two remaining VRE isolates were obtained from urine specimens. The first urine specimen was taken from a 55 year old man with interstitial lung disease and respiratory failure and complicated with cardiac event. He had been hospitalized more than one month and also had recurrent admission to intensive care unit and prolonged intubation. He was put on piperacillin-tazobactam for more than 3 weeks because the tracheal aspirate and urine grew Pseudomonas sp. The urine culture was repeated twice after the initial VRE isolation. The patient was not treated for VRE in view of the negative blood culture. Repeated urine culture for the fourth time was negative for VRE but this patient died due to severe pneumonia with respiratory failure one month later. As highlighted by the case, the risk factors that were present such as prolonged hospitalization and exposure to antimicrobial agents could predict VRE colonization. Several studies used case control methods and multivariate analysis to examine the risk factors for VRE infection in hospitalized patients. Among the risk factors that emerged were longer duration of hospitalization and longer lengths of stay in ICU[9] In our study, the use other antibiotics

(Carbapenem, Cephalosporin) was not statistically significant risk factor to predict the VRE. The other urine culture which isolated grew VRE was taken from a patient with nephrotic syndrome who also had recurrent hospitalization. However repeated urine culture 3 days after revealed no growth and patient was well. The 6 th VRE isolate grew from a culture of tissue scrapings taken from sacral bed sores. The patient is a 68 year-old diabetic Chinese man with Parkinsonism who was admitted for severe pneumonia that required ventilation. He had been in ICU for more than 2 weeks and developed sacral sores. The patient expired on the day when the specimen was taken. The risk factor for this patient was vancomycin exposure for the treatment of Methicillin-Resistant Staphylococcus aureus (MRSA) in a previous admission and prolonged hospitalization. The exposure of patient's endogenous enterococcal flora to vancomycin is a known risk factor for the development of resistance. The patient might also be exposed to other antibiotics like cephalosporin group as he had history of recurrent hospitalization.

It has been shown that vancomycin use was a major risk factor for the VRE infection or colonization[10]. An increased risk of VRE infection and colonization had been associated with non-ambulatory status[11] , receipt of antibiotics and hospitalization[12]. In this study, the use of vancomycin was the significant risk factor for the development of VRE by statistical analysis. The RR (relative ratio) represents the effect of vancomycin use on promoting resistance to vancomycin in Enterococcus spp. In multivariate logistic regression analysis, prolonged hospitalization and use of vancomycin were statistically significant risk factors where the vancomycin usage gives the higher predictor for the development of VRE. Therefore, we can conclude that patients with the significant risk factors (prolonged hospitalization and vancomycin use) had 46% of getting the VRE.

In previous studies, VRE colonization was associated with prolonged length of hospital stay, previous admission to an intensive care unit (ICU), and severe underlying disease[13]. In addition, Huh et al.[14] showed evidence that longer hospitalization and ICU stay are possible risk factors to get colonized with multiple clones of VRE. Extensive or multiple hospitalizations show a correlation between the subsequent development of VRE infections, prior antibiotic treatment, prolonged stay within intensive care units and, in some instances, even intrahospital transfers[10].

Other investigators have shown that ongoing vancomycin use creates and maintains an intraluminal environment favorable to VRE growth, thus increasing the likelihood of VRE entry into bloodstream[10]. A study conducted from 2003 to 2004 showed that prior use of antimicrobial therapy, including vancomycin and cephalosporin, has been shown to be associated with acquisition of VRE[15]. Also, the presence of underlying disease was significantly associated with an increased risk for VRE colonization. Of note, impaired renal function and hemodialysis have been previously implicated as risk factors in VRE outbreaks[12]. The use of vancomycin increased the risk of colonization 2.5-fold and third generation cephalosporins 2-fold[10,11]. A study showed that vancomycin use is the major risk factor for VRE bacteremia . Since 1990s, vancomycin and ceftazidime have been used as first line empirical therapy for line related sepsis in the renal unit (HKL), however with the emergence of VRE, it has been changed to cloxacillin and ceftazidime

Other risk factors that have described in this study like cephalosporin usage, use of carbapenem, malignancy, instrumentations like catheter and previous hospitalization were not statistically significant. Edmond et al[16] studied VRE bacteremia on an oncology ward found that the use of antimicrobial agents with activity against anaerobes (metronidazoles, clindamycin and imipenem) determined a risk factor for the development of vancomycin-resistant bacteremia. In the present study, all the 6 confirmed VRE cases were further analysed by polymerase chain reaction (PCR) and all of them showed vanA phenotypes. Sixty seven percent (4/6) were identified as E. faecium and others were identified as E. avium and E. faecalis. Although E. faecalis was more common in human infections, vancomycin resistance was more frequently observed in E. faecium isolates. Most vancomycin-resistant E .faecium (VREF) strains isolated in Korea showed the VanA phenotype, which was defined as having high level resistance to vancomycin and teicoplanin[6]. There were reports of vancomycin-resistant enterococci with vanA genotype and vanB phenotype, detected in Japan, Korea and Taiwan ([17,18]. Van D-like phenotype associated with a vanA genotype, E. faecium was found in France[19]. In our study, we found vancomycin-resistant E. avium , that was isolated from a blood culture of an elderly man with end stage renal failure, exhibited lower MIC of vancomycin (84 μg/ml) compared to the other isolated VRE cases.

CONCLUSION

The findings of the study provided first Malaysian data on the prevalence of VRE and VSE in patients reported to HKL, Malaysia. Clinical features of the patients infected with enterococci infections were provided for the identification of the clinicians. The results would serve as an alert to the clinicians of the emergence of infections by VRE and VSE and encourages implementation of appropriate infection control measures in order to curb further rise in prevalence.

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