

Predictors of Vancomycin-resistant Enterococcus (VRE) Carriage in the First Major VRE Outbreak in Singapore

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Abstract

Introduction: Until recently, vancomycin-resistant enterococcus (VRE) infection or colonisation was a rare occurrence in Singapore. The first major VRE outbreak involving a 1500-bed tertiary care institution in March 2005 presented major challenges in infection control and came at high costs. This study evaluates the predictors of VRE carriage based on patients' clinical and demographic profiles. **Materials and Methods:** Study patients were selected from the hospital inpatient census population during the VRE outbreak (aged 16 years or more). Clinical information from 84 cases and 377 controls were analysed. **Results:** Significant predictors of VRE carriage included: age >65 years [Odds ratio (OR), 1.98; 95% CI (confidence interval), 1.14 to 3.43]; female gender (OR, 2.15; 95% CI, 1.27 to 3.65); history of diabetes mellitus (OR, 1.94; 95% CI, 1.14 to 3.30), and staying in a crowded communal ward (OR, 2.75; 95% CI, 1.60 to 4.74). Each additional day of recent hospital stay also posed increased risk (OR, 1.03; 95% CI, 1.01 to 1.04). **Conclusion:** Elderly diabetic females with prolonged hospitalisation in crowded communal wards formed the profile that significantly predicted VRE carriage in this major hospital-wide outbreak of VRE in Singapore. It is imperative that active VRE surveillance and appropriate infection control measures be maintained in these wards to prevent future VRE outbreaks.

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Introduction

Vancomycin-resistant enterococci (VRE) have emerged as one of the most challenging nosocomial threats in the past decade globally.^{1,2} Although antibiotic exposure may facilitate VRE transmission by providing selective advantage for VRE and increasing the concentration of VRE in the stools, antibiotic use in association with de novo development of VRE is thought to be unlikely or impossible, since spontaneous vancomycin-resistance mutations have not been observed.³ Interventions focused on preventing horizontal transmission are reported to have a greater impact in controlling the spread of VRE compared to efforts to improve antibiotic use, even if it is endemic, has a high prevalence, and is polyclonal.³⁻⁵

Although *Enterococcus* has a low virulence, it poses a grave danger to those who are immunocompromised.⁶ There is also a risk of transfer of vancomycin-resistant genes from *Enterococcus* to *Staphylococcus aureus*,^{7,8} which is a common cause of hospital- and community-acquired infections.⁹

Various studies have been done to identify possible risk factors and to evaluate different strategies of limiting VRE spread,¹⁰⁻¹⁹ but as the hospital setting, situation and work practices may vary, the vulnerabilities and outbreak management strategies may differ.

Until recently, VRE was a rare occurrence in Singapore. In early 2005, the first major outbreak of VRE occurred in a Singapore acute-care hospital with 1500 beds. The alert

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started with the detection of 6 monoclonal cases of vanB VRE from 9 to 23 March 2005. Whenever a new case was detected, the carrier would be isolated and stool screening for VRE would be performed on existing inpatients who had shared the same cubicle with the carrier during the carrier's inpatient stay. By 3 April 2005, a total of 34 new vanB VRE carriers had been identified. The hospital management made a decision to conduct a hospitalwide VRE screening and segregate all inpatients or newly admitted patients according to their known or unknown VRE status.

In attempting to contain the first major outbreak of VRE in Singapore, various strategies that other groups have adopted^{20,21} were considered. We adopted the systematic approach used by Christiansen et al²⁰ from Australia in managing the outbreak. The steps consisted of (1) forming a disease outbreak task force; (2) rapid laboratory identification using culture and polymerase chain reaction detection of vanA and vanB resistance genes; (3) mass screening of all hospitalised patients with isolation of carriers and cohorting of contacts; (4) environmental screening and increased cleaning; (5) electronic flagging of medical records of contacts; and (6) antibiotic stewardship. The SHEA guideline³ served as useful reference materials for the detailed implementation of infection control practices. This approach worked well in controlling the outbreak but was operationally onerous and highly resource-intensive. In order to explore possible alternative cost-effective solutions suited to our own circumstances subsequently, we evaluate the predictors of VRE carriage based on patients' clinical and demographic profiles in this study.

Materials and Methods

Study patients were drawn from the hospital inpatient census population (age ≥ 16 years) on 2 April 2005, during the height of the VRE outbreak. Data from 84 cases (patients with positive VRE cultures of clinical, stool or rectal swab specimens anytime between 2 April 2005 and 30 June 2005) and 377 controls (patients with at least 3 negative VRE stool or rectal swab culture specimens taken at least a day apart during the same period) were analysed in a retrospective, unmatched case-control study using forward stepwise logistic regression.

Screening for VRE was done by taking rectal swabs or stool samples from patients, and these were either directly plated onto Enterococcosel agar (Becton Dickinson and Company, Sparks, MD, USA) containing 6 mg/L vancomycin (Sigma, St Louis, MD, USA) or first inoculated into Enterococcosel broth (Becton Dickinson and Company) containing 8 mg/L vancomycin (Sigma). If the inoculated broths changed colour after overnight incubation, DNA

was extracted and subjected to real-time PCR for vanA/B genes (Roche Diagnostics GmbH, Mannheim, Germany). If the RT-PCR was positive, the broth was subcultured onto Enterococcosel agar containing 6 mg/L vancomycin.

Enterococci were identified by colony morphology, Gram stain appearance, hydrolysis of esculin in the presence of 40% bile salts, and growth in 6.5% NaCl broth.²² Suspect VRE cases were further confirmed by Vitek 2 GP identification cards (bioMérieux, Marcy-l'Etoile, France).

Susceptibility testing was done using Vitek 2 AST P535 cards and confirmed by Etest (AB Biodisk, Solna, Sweden) if required. When necessary, RT-PCR for vanA/vanB genes was also performed on a culture extract to confirm if the van genotype correlated with the resistance phenotype. All VRE isolated underwent molecular typing by pulsed-field gel electrophoresis (PFGE), following the protocol of Oon et al.²³

Data were extracted from patients' medical records and clinical, administrative, and laboratory computerised databases. Basic demographic and health data were recorded, including age, gender, ethnicity, nationality, medical history of number of comorbidities (excluding hypertension and dyslipidaemia), diabetes mellitus, haematological or solid malignancy, chronic renal failure not requiring dialysis, or end-stage renal failure requiring dialysis and type of dialysis. Hospitalisation characteristics were also captured, such as ward classes and total length of stay in the same hospital from the beginning of the year 2005 until VRE status of patient was confirmed by either 1 positive result or 3 consecutive negative screens. The different classes of ward are differentiated by the number of patient-occupants per room. A-class wards consist of single rooms, while B-class wards accommodate 4 to 5 patients per room, and C-class wards consist of 9 bedded rooms.

Characteristics of the VRE were also captured, such as resistance phenotypes, enterococcal species, PFGE clonal patterns, and if these carriers were colonised or infected cases at the point of diagnostic confirmation.

The hospital institutional review board approved the study protocol. Statistical analyses were performed using SPSS 13. For bivariate analysis, the categorical variables were analysed using Fisher's exact or Chi-square tests. Lengths of stay in days were compared using the Mann-Whitney U test. Unconditional multiple logistic regression was used for multivariate analysis using the forward stepwise procedure.

Results

Eighty-four per cent (912/1086) of the hospital census population (age ≥ 16 years) present on 3 April 2005 were

screened for VRE at least once between 3 April and 30 June 2005. Of these, 9.2% (84/912) were found to be VRE carriers (cases). Three hundred and seventy-seven patients (controls) tested negative on at least 3 or more occasions over the same period, whether during the same admission, a repeat admission, or in the outpatient clinic.

Among the sampled subjects ($n=912$), 49.1% (230/461) were female. The mean age was 63 years (SD, 15.5; range, 16 to 95). Of the subjects, 74.8% (345/461) were Chinese, 12.4% (57/461) were Malays, 9.3% (43/461) were Indians, and 3.5% (16/461) belonged to other ethnic groups. Of the subjects, 98.9% (456/461) were Singaporeans, while the rest were of other nationalities.

As shown in Table 1, of the 84 VRE carriers, there was 1 vanA *E. faecalis*, 1 vanB *E. faecalis*, and 82 vanB *E. faecium*. Three patients were found to have clinical infection from VRE. Ninety-nine per cent (81/82) of the vanB *E. faecium* isolates were subjected to PFGE. Four distinctive patterns were identified, with a predominant clone accounting for 79% (64/81) of all vanB *E. faecium* strains.

Table 2 shows the results of the bivariate and multivariate analyses. Using forward stepwise logistic regression analysis: age of 65 years and above, female gender, medical history of diabetes mellitus and being hospitalised in C-class hospital wards (a relatively crowded ward) were found to be significant predictors of VRE carriage.

Every additional day of hospital stay also posed added risk, with odds ratio (OR) of 1.03, (95% CI, 1.01 to 1.04). Being warded in a C-class ward posed the highest risk, with OR of 2.75 (95% CI, 1.60 to 4.74). The presence of haematological malignancy appeared to confer a protective effect, with OR of 0.12 (95% CI, 0.02 to 0.91).

Calibration of the logistic regression model was tested with the Hosmer-Lemeshow test and the *P* value was 0.67,

implying good ability of the model to match the predicted and observed VRE carriers across the entire spread of the data. The area under the receiver operating curve (ROC) was 0.76, (95% CI, 0.71 to 0.82) and reflects the model's good predictive accuracy.

Discussion

Geriatric diabetic females with prolonged hospitalisation in crowded communal wards formed the profile that significantly predicted VRE carriage in this first hospital-wide outbreak of VRE in Singapore.

Elderly patients are more often in need of frequent or prolonged nursing care from multiple healthcare workers than younger patients. One possible explanation for the observed results may be that the gender bias could be related to the frequent use of diapers among female patients, as this may facilitate greater ease of cross-contamination in crowded communal female wards. Male patients, on the other hand, need to be diapered less often due to the use of urosheaths.

Relative overcrowding in communal ward and longer hospitalisation in recent months increases the potential for contact and cross-contamination with other carriers. Diabetic patients were found to be at higher risk of VRE carriage in this study. This may be due to real physiological tendencies in diabetic patients. As haematological patients in this hospital are segregated from other disciplines irrespective of ward class statuses, we postulate the reduction in patient mixture may have helped to reduce transmission.

The dependent variables were extracted with accuracy because the hospital records were fully computerised. The cases and controls were selected from a large cross-sectional pool of inpatients in a tertiary, multi-disciplinary hospital and hence, the findings could be generalised beyond isolated subspecialty disciplines. The potential weakness of this

Table 1. Characteristics of Vancomycin-resistant Enterococci (VRE) Isolated

Resistance phenotypes	Enterococcus species	PFGE clonal patterns	Colonised*	Infected†	Total
vanA	<i>E. faecalis</i>	Isolated clone	1	0	1
vanB	<i>E. faecalis</i>	Not analysed	1	0	1
	<i>E. faecium</i>	Not analysed	1	0	1
		A	62	2	64
		B	5	1	6
		C	10	0	10
		D	1	0	1
Total			81	3	84

PFGE: pulsed-field gel electrophoresis

* Colonised = patients with positive culture from either clinical or stool specimen but without an associated clinical infection documented.

† Infected = patients with positive culture from clinical specimen, associated with documented clinical infection.

Table 2. Predictors of VRE Carriage Based on Patients' Clinical and Demographic Profile

Risk factors	No. (%) of		Bivariate analysis			Multivariate analysis		
	Cases (n = 84)	Controls (n = 377)	OR	95% CI	P	Adjusted OR	95% CI	P
Demographics								
Age >65 years*	52 (62)	172 (46)	1.94	1.19-3.15	0.01	1.98	1.14-3.43	0.02
Gender, female*	52 (62)	178 (47)	1.82	1.12-2.95	0.02	2.15	1.27-3.65	0.01
Ethnicity†				0.30	**			
Chinese	65 (77)	280 (74)						
Malay	11 (13)	46 (12)						
Indian	8 (10)	35 (9)						
Others	0 (0)	16 (4)						
Hospitalisation history								
C-class ward*	54 (64)	151 (40)	2.69	1.65-4.40	0.00	2.75	1.60-4.74	0.00
Length of stay (days)‡ §	32 (median)	21 (median)			0.00	1.03	1.01-1.04	0.00
Medical history*								
Diabetes	48 (57)	138 (37)	2.31	1.43-3.73	0.00	1.94	1.14-3.30	0.01
Haematological malignancy	1 (1)	40 (11)	0.10	0.01-0.75	0.00	0.12	0.02-0.91	0.04
Solid malignancy	9 (11)	76 (20)	0.48	0.23-0.99	0.04	**		
Chronic renal failure not requiring dialysis	12 (14)	36 (10)	1.58	0.78-3.18	0.23	**		
End-stage renal failure requiring dialysis	22 (26)	68 (18)	1.61	0.93-2.80	0.10	**		
3 or more co-morbidities, excluding hypertension and dyslipidaemia	67 (80)	219 (58)	2.84	1.61-5.03	0.00	**		

95% CI: 95% confidence interval; OR: odds ratio; VRE: vancomycin-resistant enterococcus

* Fisher's exact test; † Chi-square test; ‡ Mann-Whitney U test

§ Length of stay (in days) from 1 January 2005 until VRE status of patient is confirmed by either one positive result or three negative screening results.

|| C-class ward consists of 9-bedded rooms

** Variables not selected in forward stepwise logistic regression analysis

study was that it did not examine the influence of recent antibiotic usage on VRE carriage status as a result of resource constraint in having to establish accurately for all patients, forms and types of antibiotic usage beyond the confines of the hospital over the defined period. Despite this shortcoming, the predictive model showed adequate calibration and discriminatory accuracy for us to better understand accurately, the epidemiology of VRE carriage in local hospital setting.

Conclusion

Risk identification of VRE carriage based on patients' clinical and demographic profile appears intuitive and may be useful for making decisions on who to screen for VRE carriage and for implementing selective control measures.

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