Vancomycin resistant enterococcal (VRE) colonization among patients treated in intensive care units at the National Hospital of Sri Lanka, and determination of genotype/s responsible for resistance

C Kannangara[1], **P Chandrasiri**[1], **E M Corea**[2]

(Index words: vancomycin resistance, enterococci, rectal colonization, phenotype, genotype)

Abstract

Background The aim of this study was to assess the epidemiology of VRE colonization among patients in the intensive care units (ICU) of the National Hospital of Sri Lanka (NHSL).

Methods A cross sectional study was carried out on 218 patients admitted to 12 ICUs of the NHSL from January to March 2012. Rectal swabs were collected on day 0, 4, 8 and every 4th day thereafter till discharge. Enterococci were isolated on selective media and identified up to species level using standard bacteriological procedures. Standardized disc diffusion antibiotic susceptibility testing to ampicillin, teicoplanin and vancomycin was performed using the Clinical and Laboratory Standards Institute (CLSI) method. Minimum inhibitory concentrations to vancomycin were determined, using the E-test in strains showing intermediate or frank resistance to vancomycin by disc diffusion. Genotype determination *(van A / van B)* was carried out on isolates identified as VRE using the polymerase chain reaction. Patients positive for VRE colonization were followed up to discharge or death.

Results VRE prevalence in the study sample was 5%. Univariate analysis showed that the use of metronidazole (odds ratio [OR]: 15.73; 95% 95% confidence interval [CI]: 3.94-62.67, P<0.05) or teicoplanin (OR: 12.56; 95% CI: 2.65 - 59.52, p<0.05) and diabetes (OR: 05.13; 95% CI: 1.36 - 18.7, p<0.05) or hemodialysis during ICU stay (OR: 7.38; 95% CI: 1.69-32.16, P<0.05) were associated with an increased risk of VRE colonization.

Conclusion The 5% prevalence of VRE colonization detected signals the emergence of VRE in the intensive care setting in Sri Lanka.

Ceylon Medical Journal 2018; **63:** 154-158

DOI: http://doi.org/10.4038/cmj.v63i4.8766

Introduction

Enterococci are part of the normal intestinal flora of humans and animals. Although usually sensitive to penicillin and ampicillin, they are intrinsically resistant to most other groups of antibiotics, including widely used drugs such as cephalosporins and aminoglycosides. They also can acquire resistance to the penicillin group of antibiotics, and glycopeptides. This has serious implications for treatment of patients infected with these organisms and for infection control. Vancomycin-resistant enterococci (VRE) were first reported in the United Kingdom, Europe and the United States in 1986, and then became widespread [1].

Three glycopeptide resistant phenotypes can be distinguished based on the level and inducibility of resistance to vancomycin and teicoplanin [2]. The VanA type is characterized by acquired inducible resistance to both vancomycin and teicoplanin [3]. Strains of the VanB type have acquired inducible resistance to various levels of vancomycin but not to teicoplanin [4]. Constitutive low-level resistance to vancomycin (VanC type) is an intrinsic property of motile enterococci, *E. gallinarum* [5], *E. casseliflavus* [6] and *E. flavescens* [7].

Seven different glycopeptide resistant genotypes have been described. Five of them (*vanA, vanB, vanD, vanE* and *vanG*) are acquired while two of them (*vanC1* and *van C2/C3*) are intrinsic. The most common resistant genotypes are *vanA* and *vanB* [8]. Resistant genes are located on conjugative plasmids or transposons [9].

Colonization of the gastrointestinal tract is a prerequisite to infection. Rectal colonization with VRE is a more important predictor of infection than colonization of the rest of the gastrointestinal tract [10,11].

Due to the presence of multiple risk factors, the prevalence of VRE in the ICU setting is expected to be

[1]*National Hospital of Sri Lanka,* [2]*University of Colombo, Sri Lanka.*

Correspondence: CK, *e-mail: <chiranthikannangara@gmail.com>.* *Received 24 August 2018 and revised version accepted 03 October 2018.*

much higher than in other hospital settings. Between 1989 and 1993, the incidence of VRE colonization among patients admitted to intensive care units (ICUs) in the USA increased from 0.4% to 13.6%, representing a 34-fold increase [13]. Early identification of VRE colonization in high-risk patients is important for the prevention and control of hospital acquired VRE infections [14]. If surveillance cultures are not performed, asymptomatic carriage can be easily missed. Standard culture methods and molecular techniques like PCR are used for surveillance of VRE colonization.

The major reasons for performing surveillance are to determine the size of the problem, and to determine whether any particular type of resistance is spreading or is associated with an outbreak. Such an evaluation has not been performed in Sri Lanka since 2007 [15].

Objectives

In this study, we investigated the prevalence of VRE rectal colonization and the genetic mechanism of glycopeptide resistance in VRE isolates in patients in ICUs of the National Hospital of Sri Lanka (NHSL). The association between several risk factors and rectal colonization with VRE was investigated, and the relationship between VRE colonization, VRE bactaeraemia and patient survival was assessed.

Methodology

This was a descriptive study conducted on 218 patients admitted to 12 selected ICUs and high dependency units (HDU) of the NHSL from January to March, 2012.

Sample size was calculated using the formula n=Zp (1-p)/d [16]. In a previous study done in Sri Lanka in 2007, the prevalence of VRE was 0.013 [17]. We calculated the sample size with an absolute error of 1.5% and 5% α error. Therefore, the required sample size was 218.

Patients already identified as VRE colonized on a previous admission and patients on whom a rectal swab was contraindicated (e.g. those with a bleeding tendency) were excluded from the study.

Data collection and variables

Data on demographic characteristics and associating factors were collected using an interviewer-filled questionnaire and by reviewing patients'medical records. Data collected included the following; demographics, comorbidities including medical and surgical conditions (hypertension, malignancy, type of surgeries, diabetes, ischaemic heart disease, chronic kidney disease, obstructive airway disease, acute renal failure, use of immunosuppressants, and chronic liver cell disease), duration of hospital stay in hours prior to admission, exposure to antibiotics during last 60 days, and ICU admissions within last 6 months and interventions performed during ICU stay.

Sample collection and processing

Two rectal swabs were taken within 24 hours of admission to ICU. Thereafter, two rectal swabs were obtained on day 4, day 8 and every 4th day till discharge. The swabs were inoculated on Enterococcosal agar plates containing 6µg/ml vancomycin and 6µg/ml ampicillin, respectively. After 24 hours and 48 hours of incubation, aesculin positive (magenta) colonies were sub-cultured on blood agar plates.

Identification of Enterococcus species

Isolates were identified as *Enterococcus* spp. by Gram stain, catalase test and the PYRase test. They were tested for the presence of motility and pigment production to exclude *E. gallinarum* and *E. casseliflavus*. Detection of acid production from pyruvate and arabinose to distinguish *E. faecalis* (pyruvate positive) and *E. faecium* (arabinose positive) was carried out only on the non-motile, non-pigmented isolates.

Antibiotic sensitivity testing

Antibiotic sensitivity testing (ABST) of all *E. faecalis* and *E. faecium* isolates to ampicillin, vancomycin and teicoplanin was done according to the Clinical Laboratory Standard Institute (CLSI) disc diffusion method. In strains showing intermediate or frank resistance, minimum inhibitory concentrations (MIC) of vancomycin were determined by E-testing. MIC values >32 µg/ml were considered a resistant according to CLSI guideline 2011 [18].

Detection of vanA and vanB genes

Genotype determination of *vanA* and *vanB* was carried out on isolates identified as VRE using conventional PCR.

Statistical methods

Pearson Chi square test, (or Fisher exact test) was performed for data analysis. Odds ratios and 95% confidence intervals (CIs) were used to assess risk. P values below 0.05 were considered significant.

Ethics approval

Ethics approval was obtained from the Ethics Review Committee, NHSL. Informed written consent was obtained from the patient or, in the case of patients less than 18 years and in patients who could not give consent, from the guardian.

Results

A total of 288 duplicate (i.e. 576) rectal swabs were collected from the 218 patients over their period of stay in ICU. Out of the two swabs taken, one was inoculated on

ampicillin containing media while the other was inoculated on vancomycin containing media. There were 104 enterococcal growths in ampicillin-containing media and 74 enterococcal growths in vancomycin-containing media, giving a total of 178 enteroccocal growths belonging to 101 patients.

Species identification

Of the 178 isolates, 118 isolates (from 80 patients) were motile *E. gallinarum*. No pigment producing *E. casseliflavus* were isolated. Of the remaining isolates, 57 were non-motile and 3 were classified as having sluggish motility. These 60 isolates, from 21 patients, were further tested with pyruvate and arabinose fermentation tests. 50 isolates were identified as *E. faecium* (from 13 patients) and 7 isolates were identified as *E. faecalis* (from 6 patients). The three isolates with uncertain motility did not show pyruvate or arabinose fermentation (Figure 1).

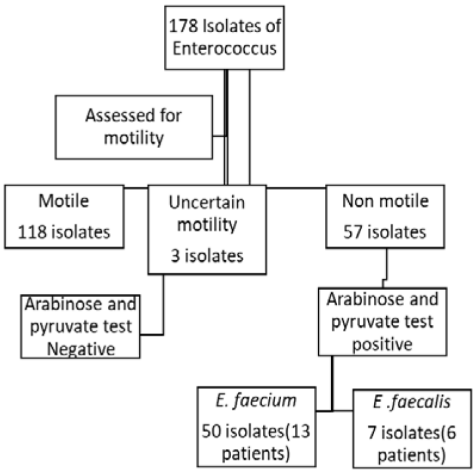

Figure 1. **Identification of *E.faecium* and *E. faecalis.***

Antibiotic sensitivity testing

The 57 *E. faecalis* and *E. faecium* isolates were assessed for vancomycin, ampicillin, and teicoplanin sensitivity using the CLSI disk diffusion method. Of the 57 enterococcal isolates, 35 isolates showed resistance or intermediate resistance to vancomycin in the disk diffusion method. These 35 isolates were further tested with E-strips to determine vancomycin MICs (Table 1). Twenty-two (22) isolates from 11 patients showed MICs >32µg/ml.

Table 1. MIC of the 35 isolates that showed resistance or intermediate resistance to vancomycin by the CLSI disk diffusion method

MIC value (µg/ml)	*Total isolates*	*Number of patient's (%)*
>32	22	11 (69%)
8-16	9	4 (25%)
<4	4	1 (6%)
Total	35	16

VRE colonisation

Prevalence of VRE rectal colonization in the study sample was 5% (11/218). Four patients were colonized with VRE on admission to ICU. Therefore, the prevalence of VRE colonization on admission to ICU was 2% (4/218). Two of these were direct admissions to ICU from community. Seven patients acquired VRE rectal colonization during ICU stay. Prevalence of VRE acquisition during ICU stay was 3% (7/214).

Among the 11 VRE isolates characterized in this study, all isolates were *E. faecium* with high-level resistance to vancomycin (MICs 32-512 mg/L) and resistance to teicoplanin which is characteristic of the *vanA* phenotype. On PCR testing, all of them carried the *vanA* gene.

Table 2. Factors associated with VRE colonisation (Total 218 patients)

Characteristics	*Patients with VRE (n=11)*	*Patients without VRE (n=207)*	*P value*	*OR (95% CI)*
Mean age	55.45 years	46.8 years	0.067	
Sex (Male)	6	139	0.392	0.58 (0.17-1.99)
Prior hospital stay>48h	4.3	5.6	0.862	0.89 (0.25-3.15)
Antibiotic use within last 60 days	9	146	0.428	1.88 (0.39 to 8.95)
Diabetes	4	21	0.015	5.06 (1.63-18.73)
Immunosuppressed states	0	08	0.989	1.02 (0.05-18.79)
Chronic kidney disease	01	10	0.536	1.97 (0.22-16.93)
Prior ICU admissions*	0	0		
Nasogastric tube	2	95	0.091	0.26 (0.05-1.24)
Lower GI Procedure	0	05	0.755	1.60 (0.08-30.75)
HD during ICU Stay	03	10	0.007	7.35 (1.69-32.16)
Surgery this admission**	07	97	0.292	1.98 (0.56-6.98)
Invasive ventilation	08	112	0.243	2.26 (0.58-8.76)
Shock needing inotropes	08	149	0.977	1.03 (0.26-3.98)
Use of antibiotics:				
Metronidazole	08	30	0.0001	15.64 (3.94-62.67)
Teicoplanin	03	06	0.001	12.56 (2.65-59.23)

(OR: odds ratio; CI: confidence interval; VRE: vancomycin resistant enterococci; * Prior ICU admissions within last 6 months, ** gastro intestinal or abdominal surgery in current admission)

The presence of diabetes was found to be significantly associated with VRE colonisation (OR: 05.06; 95% CI: 1.36-18.73, p = 0.015). Twenty-seven percent of VRE positive patients had hemodialysis during ICU stay compared with 10 (4.8%) in the non VRE group (OR: 7.35; 95% CI: 1.69-32.16, p = 0.007). Use of teicoplanin (OR: 12.56; 95% CI: 2.65 - 59.52, p = 0.001) and metronidazole (OR: 15.64; 95% CI: 3.94-62.67, P = 0.0001) were found to be associated with VRE rectal colonization. Use of vancomycin was not associated with VRE colonization in this study.

Colonisation with other resistant strains

The prevalence of ampicillin resistant enterococcal rectal colonization and teicoplanin resistant enterococcal rectal colonization in the study sample was 8.2% (18/218) and 5% (11/218) respectively.

Outcome of colonisation

None of the VRE rectal colonized patients developed VRE bacteraemia or infection with VRE. Mortality was 17% (n = 37) in the total sample. There was no significant difference between the mortality rates of VRE colonized group and non-colonized group.

Discussion

We like to highlight the fact that this study was conducted in 2012 and the data may not represent the current situation in the country. The VRE rectal colonization prevalence of 5% among patients in ICUs at NHSL found in this study is comparable to the prevalence values seen in other studies in Asia (5%) (19). However, it contrasts with the absence of VRE colonisation in a similar study done in NHSL in burns unit and orthopaedic units in 2004 [16]. This difference could be due to a true increase in the prevalence of VRE over the preceding 8 years or could be due to differences in the study settings.

In the current study the presence of diabetes, haemodialysis and use of metronidazole and teicoplanin showed a significant association with VRE colonization while age, gender, duration of hospitalization, and prior ICU admission were not significant risk factors for VRE colonization. Although previous vancomycin use has been considered to be a risk factor for VRE colonization, the current study did not clearly show such an association. This is somewhat surprising. Other underlying conditions such as septicemia, immunosuppression, neutropenia, malignancy, cardiac disease, and major surgery did not show an association with VRE colonization. However, it is possible that the study population was not large enough to determine risk factors for colonization with VRE.

In this study, 4 VRE positive patients were VRE positive on admission to ICU of whom two were direct admissions from the community. This indicates community carriage of VRE. This is of great concern as VRE may give rise to difficult to treat community acquired infections in healthy persons.

All 22 VRE isolates (from 11 patients) identified in this study, were *E. faecium* with high-level resistance to vancomycin (MICs 32-512 mg/L) and teicoplanin which is characteristic of the *vanA* phenotype. All of them (100%) carried the *vanA* gene. A similar predominance of *E. faecium* has been observed in studies on nosocomial VRE infections in India [14]. This finding is of clinical importance since *E. faecium* is more resistant to antibiotics than *E. faecalis*, thus limiting the therapeutic options.

The high rate of ampicillin (30%) and teicoplanin (5%) resistance found in this study is worrying as infections with these strains will require increased use of vancomycin which may drive the emergence of further strains of VRE.

Conclusions

VRE colonization has emerged in Sri Lanka but is still uncommon (5%), even in the ICU setting in 2012. However, both community and nosocomial acquisition of VRE was found. The predominant strain was *vanA* positive *E. faecium* with high level vancomycin resistance. Risk factors that showed a significant association for colonization with VRE include diabetes, hemodialysis during ICU stay and use of metronidazole and teicoplanin. Ampicillin resistance in enterococci was also common (30%) in this setting.

Recommendations

As VRE carriage is low and infection due to VRE is uncommon, routine screening for VRE is probably not cost effective. The role of community acquired VRE should be studied. Larger case control studies to further elucidate the risk factors for VRE colonization and infection should be conducted. As modifiable risk factors have not been identified an increased emphasis on adherence to standard infection control precautions may prevent spread. Further studies are necessary to assess the current VRE colonization and infection prevalence in the country.

Acknowledgements

We sincerely thank Prof Jennifer Perera, Professor of Microbiology, Department of Microbiology, Faculty of Medicine, University of Colombo and School of Pathology and Professor Tim Inglis, Professor Pathology and Laboratory medicine, UWA, Path West Laboratory Medicine for the assistance provided for PCR work. Special thanks go to all Consultant Anaesthetists and Physicians who granted permission to access the ICUs.

Competing interests

The authors declare no competing interests.

References

1. Uttley AH, Collins CH, Naidoo J, George RC. Vancomycin-resistant enterococci. *Lancet* 1988; **1**(8575-6): 57-8.
2. Dutka-Malen, Evers S, Courvalin P. Detection of Glycopeptide Resistance Genotypes and Identification to the Species Level of Clinically Relevant Enterococci by PCR. *J Clin Microbiol* 1995; 24-27.
3. Washington W, Stephen A, William J, *et al*. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins, 2006.
4. Quintiliani R Jr, Evers S, Courvalin P. The vanB gene confers various levels of self-transferable resistance to vancomycin in enterococci. *J Infect Dis* 1993; **167**(5): 1220-23.
5. Leclercq R, Dutka-Malen S, Duval J, Courvalin P. Vancomycin resistance gene *vanC* is specific to *Enterococcus gallinarum*. *Antimicrob*. *Agents Chemother* 1992; **36**: 2005-8.
6. Vincent S, Knight RG, Green M, Sahm DF. Vancomycin susceptibility and identification of motile enterococci. *J. Clin Microbiol* 1991; **29**: 2335-7.
7. Navarro F, Courvalin P. Analysis of genes encoding D-alanine: Dalanine ligase-related enzymes in *Enterococcus casseliflavus* and *Enterococcus flavescens*. *Antimicrob*. *Agents Chemother* 1994; **38**: 1788-93.
8. Perez-Hernandez X, Mendez-Alvarez S, Claverie-Martin F. A PCR assay for rapid detection of vancomycin-resistant enterococci. *Diagn Microbiol Infect Dis* 2002; **42**: 273-7.
9. Radu S, Toosa H, Rahim RA, *et al*. Occurrence of the *vanA* and v*anC2/C3* genes in *Enterococcus* species isolated from poultry sources in Malaysia. *Diagn Microbiol Infect. Dis* 2001; **39**: 145-53.
10. Hendrix CW, Hammond JMJ, Swoboda SM, *et al*. Surveillance Strategies and Impact of Vancomycin-Resistant Enterococcal Colonization and Infection in Critically Ill Patients. *Ann Surg* 2001; **233**(2): 259-65.
11. Leclercq R, Courvalin P. Resistance to glycopeptides in enterococci. *Clinical Infectious Diseases* 1997; **24.**4: 545-54.
12. Milestone A, Maragakis L, Conroll K, Perl T. Targeted Surveillance to Identify Children Colonized with Vancomycin Resistant Enterococcus in the Paediatric Intensive Care Unit. *Inf. Contr. Hosp. Epidemiol* 2010; **31**(1): 94-7.
13. Centres for Diseases Control. Nosocomial enterococci resistant to vancomycin – United States 1989-1993. *MMWR* 1993; **42**: 597-9.
14. Robert C, Moellering JR. *Enterococcus* species, *Streptococcus bovis* and *Leuconostoc* species. In: Mandell GL, Bennett JE, Dolin R (eds). Principles and Practice of Infectious Diseases. Sixth edition, New York. Churchill Livingstone 2005: 2411-21.
15. Gunasekera S, Perera J. Drug resistant enterococci: factors associated with gastrointestinal tract colonization. *Ceylon Journal of Medical Science* 2008; **50**(1): 9-14.
16. Lwanga SK, Lemeshow S, World Health Organization. Sample size determination in health studies: a practical manual. Geneva: World Health Organization. 1991.
17. Perera S, Meegoda R, Janapriya R. Fernando N. Meedin DFD. A study to detect vancomycin resistant enterocooci among clinical isolates at high risk wards of Sri Jayawardenapura General Hospital. Annual Sessions of the College of Microbiologists, September 2006.
18. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI document M100. Clinical and Laboratory Standards Institute; 2011.
19. Ziakas PD, Thapa R, Rice LB, Mylonakis E. Trends and significance of VRE colonization in the ICU: a meta-analysis of published studies. *PLoS One* 2013; **8**(9) e75658.