

Research article

Epidemiology of invasive infections caused by vancomycin sensitive and resistant enterococcal strains among oncology patients at the National Cancer Institute of Sri Lanka from 1st of July 2012 to 31st of July 2013

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Abstract

Introduction and Objectives: Enterococci have traditionally been regarded as low grade pathogens but have emerged as an increasingly important cause of nosocomial infections in the last decade. While the *Enterococcus faecalis* remains the predominate species in clinical infection, *Enterococcus faecium* isolates are increasing in proportion. This study was carried out to describe the epidemiology of invasive enterococcal infections among oncology patients at the National Cancer Institute of Sri Lanka (NCISL).

Methods: 60 patients with invasive enterococcal infections, who were treated as inward patients at the National Cancer Institute of Sri Lanka from 1.7.2012 to 31.7.2013 whose samples were sent for microbiological investigation were included in this study. Speciation of the isolates was done by using a rapid manual analytic system (RapID STR panel-Oxoid). Vancomycin sensitivity was assessed in all enterococcal isolates by disc diffusion method, agar dilution screening method (CLSI guideline 2013)¹⁶ and detection of minimum inhibitory concentration (MIC) by using Vancomycin gradient strips. Teicoplanin MIC was assessed only in vancomycin resistant isolates using teicoplanin gradient strips. Associated factors for getting an infection with vancomycin resistant strains were assessed using a data extraction sheet.


Results: The incidence of invasive enterococcal infections among oncology patients was 1.1 per 1000 admissions. The incidence of invasive enterococcal infection caused by vancomycin resistant species was 0.16 per 1000 admissions. *E. faecium* was the dominant species causing invasive enterococcal infections (55%). Using the gradient strip method for determination of vancomycin MIC as the gold standard, the screening agar dilution method had 100% sensitivity and specificity and the disc diffusion test had a low specificity (77.8%). Almost all the participants (96.6%) had acquired the enterococcal infection from the hospital. Vancomycin resistant infections were more common in patients who had haematological malignancies, who were treated with 3rd generation cephalosporins and cytotoxic chemotherapy drugs.

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Conclusions: *E. faecium* is the dominant species causing invasive enterococcal infections in oncology patients (55%) and phenotypically compatible with the VanA phenotype of glycopeptide resistant enterococci.

Keywords: Enterococci, vancomycin, resistant, oncology, chemotherapy

Introduction and Objectives

Although about a dozen *Enterococcus* species have been identified, *E. faecalis* and *E. faecium* are responsible for most human infections. Historically, the ratio of infections due to *E. faecalis* to those due to all other *Enterococcus* species was approximately 10:1.¹ In recent years, there has been a progressive decline in this ratio. This microbiological shift is likely to be explained in part by the emergence of vancomycin resistant enterococci (VRE) and *E. faecium* being the dominant species identified among VRE.²

The most common nosocomial infections caused by these organisms are urinary tract infections followed by intra-abdominal infections, pelvic infections, surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis. A major reason why these organisms survive in the hospital environment is due to a high level of intrinsic resistance to many antibiotic groups (β lactams, low concentrations of aminoglycosides, nalidixic acid, clindamycin, fluoroquinolones and trimethoprim-sulfamethoxazole).³ They are also able to acquire resistance to many commonly used antibiotics (glycopeptides, tetracyclines, erythromycin, fluoroquinolones, rifampicin, chloramphenicol, fusidic acid, nitrofurantoin).^{4,5}

Vancomycin resistant enterococci are classified according to their phenotype using vancomycin and teicoplanin MIC⁶ and genotype using the specific ligase gene.⁷ There are 5 recognized phenotypes (VanA, VanB, VanC, VanD, VanE) and 7 genotypes (VanA, VanB, VanC, VanD, VanE, VanG, VanL). Motile enterococci, *E. gallinarum* and *E. casseliflavus* have an intrinsically low level of resistance to vancomycin and belong to the VanD genotype.⁷ These two species have been identified as a clinically significant cause of bacteraemia in immunocompromised patients.⁸ Most of the risk factors for infection with VRE, namely immunosuppression, advanced age, other co-morbidities such as renal and liver failure, invasive procedures and devices and gastrointestinal surgery are present in oncology patients.⁷ Use of third generation cephalosporins, carbapenems and glycopeptides are also regarded as risk factors for acquisition of VRE and other glycopeptide resistant enterococci.⁷ These antibiotics are frequently used as empirical treatment for febrile neutropenia in oncology patients (NCISL antibiotic policy). No study has been done in Sri Lanka to describe the epidemiology of vancomycin sensitive and resistant enterococcal infection in oncology patients or screening of patients to evaluate the prevalence of colonization with VRE. This study was therefore carried out to describe invasive enterococcal infections caused by vancomycin sensitive and resistant strains among oncology patients at the National Cancer Institute of Sri Lanka.

The objectives of the current study were to describe the following characteristics of *Enterococcus spp.* isolated from patients with invasive infections in hospitalized oncology patients at National Cancer Institute, Sri Lanka.

- a. Determining vancomycin sensitivity
- b. Speciation of isolates
- c. Identifying factors associated with VRE infections
- d. Assess teicoplanin sensitivity of VRE isolates.

Methods

This study was conducted as a descriptive cross sectional study from 1.7. 2012 to 31.7.2013.

Sixty patients with invasive enterococcal infections were included in this study. A case of invasive enterococcal infection was defined by the isolation of *Enterococcus* spp. from a normally sterile body site. Sterile sites include blood, peritoneal aspirates, pus aspirates from abscesses and sterile body cavities, aspirate from biliary tract, pleural aspirates and cerebrospinal fluid. Urine isolates were also included if there was evidence of pyelonephritis. Nosocomial infection was defined as infection obtained >72 hr. after hospital admission.

The sample size of patients with invasive enterococcal infection necessary to obtain the required precision (0.3) and confidence (95%) level was 60.

A pilot study was done from 1.1.11 to 31.7.2011 using laboratory work sheets to determine the prevalence of invasive enterococcal infection in oncology patients at NCISL and prevalence was calculated as 0.7%. Using this prevalence, the required sample size of VRE to obtain a precision of 0.3 and confidence level of 95% was determined to be 60 which could be expected from a total sample size of 8570. During the study period 9652 samples were processed which included 4051 blood cultures, 4652 urine cultures, 855 pus aspirates, 35 pleural fluid samples, 36 peritoneal fluid samples and 23 cerebrospinal fluid samples.

Enterococcal blood stream infection was defined as the isolation of an *Enterococcus spp* from one peripheral blood culture sample. When blood cultures from both central catheter and peripheral site were collected at the same time, if blood culture through the catheter became positive two or more hours before peripheral blood culture, it was considered as catheter associated blood stream infection. Enterococcal bacteremia occurring >60days after a previous episode was considered to be a separate blood stream infection. In urine cultures, a pure growth of $\geq 10^5$ colony forming units (CFU)/ml in patients with symptoms suggestive of pyelonephritis (fever with chills and rigors with loin tenderness) or patients with ultra sound scan evidence suggestive of pyelonephritis only were included in this study.

Gram stain was performed on colonies which had growth on both blood and MacConkey agar (without salt) and which were catalase negative. In urine cultures, Gram stain was performed on lactose fermenting catalase negative colonies on CLED medium. Gram positive cocci in chains were Lancefield grouped. Lancefield group D isolates were further tested for growth on 6.5% NaCl, survival at 60 °C for 30 minutes, and hydrolyzing of L-pyrrolidonyl-b-naphthylamide (PYR) to identify enterococci. All isolates which showed growth on 6.5% NaCl, a positive result for PYR and survived 30 minutes at 60 °C were included in the study.⁹

Speciation of isolates was done using a rapid manual analytic system (Rapid STR panel-Oxoid) according to the manufacture instructions. Rap ID STR panels contain 10 reaction cavities for different biochemicals and test cavities seven through ten are bifunctional. In addition to these biochemical tests, haemolysis on blood agar plate was assessed according to the manufacturer's instructions. The microcode obtained from the test kit was compared with the rapid STR code compendium for the identification. Evaluation of motility and pigment production was also done to identify motile and pigment producing enterococci.

Associated factors for getting an infection with VRE, namely prior administration of antibiotics and chemotherapy, invasive procedures and devices, gastrointestinal surgery, renal and liver failure, diabetes mellitus and mucositis, were assessed using a data extraction sheet. Data was obtained by reviewing medical records of each study patient.

Three methods, disk diffusion method using 30 µg vancomycin discs (Oxoid), agar dilution screening method and minimum inhibitory concentration (MIC) determination using gradient strip method (Oxoid vancomycin MIC Evaluator) were used to detect vancomycin susceptibility. The gradient strip method (Oxoid teicoplanin MIC evaluator) were used to detect MIC of teicoplanin on vancomycin resistant isolates. The disk diffusion test and the agar dilution screening methods were performed according to the recommendations of performance standards for antimicrobial susceptibility testing, clinical laboratory standard institute, M100, 2013 (CLSI, 2013).¹⁰ According to the CLSI guideline vancomycin zone diameter criteria were ≥ 17 mm - sensitive, 15-16mm - intermediate and ≤ 14 mm - resistant. To detect vancomycin and teicoplanin MIC, the manufacturer's instructions were followed and CLSI 2013 MIC interpretive criteria were used to interpret the vancomycin and teicoplanin susceptibility. Vancomycin MIC interpretive criteria were ≤ 4 µg/ml - sensitive, 8-16 µg/ml - intermediate and ≥ 32 µg/ml - resistant. Teicoplanin MIC interpretive criteria were ≤ 8 µg/ml - sensitive, 8-16 µg/ml - intermediate and ≥ 32 µg/ml - resistant. Controls used were *E. faecalis* ATCC 29212 and ATCC 51299.¹⁰

To describe the phenotype of VRE, phenotypic classification originally described by Gold et al.¹¹ was used. VanA isolates have high MICs of vancomycin (MIC range 64–1,000 µg/ml) and teicoplanin (MIC range 16–512 µg/ml), whereas VanB isolates often have lower MICs of vancomycin (MIC range 4–1,024 µg/ml but usually on the low side) but typically susceptible to teicoplanin. Van D isolates have moderate susceptibility to both glycopeptides (vancomycin MIC range 2–32; teicoplanin MIC usually < 0.5 µg/ml) whereas VanC and VanE isolates display low level resistance to vancomycin (MIC range 0.5–4 µg/ml) and are susceptible to teicoplanin.

Results

Of the 9652 samples, there were 60 clinically and microbiologically diagnosed patients with invasive enterococcal infections.

Mean age of the participants who were in the pediatric age group and non-pediatric age group are 4.92 years (SD=3.75) and 47.7 years (SD = 18.47) respectively (Table 1).

Table 1: Frequency distribution of study participants according to selected socio demographic characteristics

Characteristic	Number (n=60)	Percentage (%)
Age		
≤12 years	13	21.7
>12 years	47	78.3
Sex		
Male	22	36.7
Female	38	63.3

During the period of this study there were 12688 new registration of patients and total number of admissions was 54027. The incidence of invasive enterococcal infections among oncology patients was 1.1 per 1000 admissions. The incidence of invasive enterococcal infection caused by vancomycin resistant spp. was 0.16 per 1000 admissions.

Table 2: Association of vancomycin sensitivity of invasive enterococcal infections according to acquisition location

Location	Vancomycin				total
	sensitive		resistant		
	No	%	No	%	
Hospital	49	84.5	9	15.5	58
Community	02	100.0	0	0.0	2

Almost all the participants (96.6%) had acquired the enterococcal infection from the hospital (Table 2). The majority of the participants were admitted in the medical wards at the time of the positive culture. The highest proportion of vancomycin resistant infections were from pediatric ICU (23.3%) followed by medical wards (17.2%) and all were hospital acquired infections (Table 3).

Table 3: Association of vancomycin sensitivity of invasive enterococcus infection with location at the time of positive culture

Location	Vancomycin				Total
	sensitive		resistant		
	No	%	No	%	
Medical ward	24	82.8	5	17.2	29
Pediatric ward	7	87.5	1	12.5	8
Surgical ward	10	90.9	1	9.1	11
Medical ICU	2	100.0	0	0.0	2
Pediatric ICU	4	66.7	2	23.3	6
Surgical ICU	4	100.0	0	0.0	4

Enterococci were isolated mainly from patients with UTI and with bacteraemia, with *E. faecalis* the predominant species isolated from these 2 sites as well as from pus aspirates (Table 4). 90% of infections were caused by *E. faecium* and *E. faecalis*. Almost all the infections caused by VRE were caused by *E. faecium* (88.9%). Vancomycin resistant *E. casseliflavus* was isolated from a pus aspirate of a pelvic abscess (Table 5 and 6).

Table 4: Infections caused by vancomycin sensitive and resistant *Enterococcus* species.

Infection	Vancomycin				Total
	sensitive		resistant		
	No	%	No	%	
UTI	23	82.1	5	17.9	28
Bacteraemia	15	88.2	2	11.8	17
Pelvic abscess	4	66.7	2	33.3	5
Peritoneal infection	3	100	0	0.0	3
Surgical site infection	3	100	0	0.	3
Other body sites ^a	3	75	0	25	4

^a : Infections from other body sites include a knee joint infection, sub-phrenic abscess and buttock abscess

Table 5: Frequency distribution of the *Enterococcus* species according to the vancomycin sensitivity

Infection	Vancomycin				Total
	sensitive		resistant		
	No	%	No	%	
<i>E. faecium</i>	25	75.8	8	24.2	33
<i>E. faecalis</i>	21	100.0	0	0.0	21
<i>E. durans</i>	3	100.0	0	0.0	3
<i>E. casseliflavus</i>	1	50.0	1	50.0	2
<i>E. avium</i>	1	100.0	0	0.0	1
Total	51	85.0	9	15.0	60

Table 6: Frequency distribution of vancomycin sensitive and resistant *Enterococcus* species in blood and upper UTI

Species	Vancomycin			
	sensitive		resistant	
	Blood	Urine	Blood	Urine
<i>E. faecium</i>	9	12	2	5
<i>E. faecalis</i>	5	8	0	0
<i>E. durans</i>	1	2	0	0
<i>E. casseliflavus</i>	0	1	0	0
<i>E. avium</i>	0	0	0	0

Of the invasive enterococcal infections in patients with haematological malignancies, 22.7% were caused by resistant strains, compared to only 10.5% among patient with solid tumors. 16% of patients who had been given 3rd generation cephalosporins had infections with resistant strains, while resistant strains were not isolated from patients who not treated with a cephalosporin. Around 22% of patients who had been given chemotherapy had infections with resistant strains. However, these findings were not statistically significant (Table 7).

Only 2/17 of patients with positive blood cultures complied with the necessary criteria to be identified as bacteraemia associated with an intravascular catheter. Vancomycin resistant isolates were not found in these 2 isolates although prevalence of vancomycin resistance among the patients who had a CVC was 22.2%.

Seven of the 9 patients with VRE had expired at the time of collecting data from medical records.

Using the gradient strip method as the standard, the disc diffusion method did not detect vancomycin resistance in 2 isolates, *E. faecium* and *E. casseliflavus*, with an MIC of 6µg/ml (Table 8 and 9). Using the same method, Vancomycin MIC of vancomycin sensitive strains ranged from 0.5- 2 µg/ml. MICs of resistant strains is shown in Table 10. The vancomycin resistant *E. faecium* isolates are compatible with VanA phenotype. Of the 2 *Enterococcus casseliflavus* isolates, one had a vancomycin MIC of 6 µg/ml and teicoplanin MIC of 0.5 µg/ml. The MIC of the second isolate was 1 µg/ml (vancomycin susceptible).

Table 7: Association of the vancomycin sensitivity of invasive enterococci with patient characteristics.

Characteristic		Vancomycin				Total	Significance Fisher's exact
		sensitive		resistant			
		No	%	No	%		
Malignancy	Solid	34	89.5	4	10.5	38	P = 0.267
	Haematological	17	77.3	5	22.7		
Hospital stay ¹	Short stay	27	87.1	4	12.9	31	P = 0.727
	Long stay	24	82.8	5	82.8		
ICU admission ²	Yes	14	87.5	2	12.5	49	P = 1.00
	No	37	84.1	7	15.9		
Chemotherapy ³	Given	32	78.0	9	21.9	41	P = 0.046
	Not given	19	100.0	0	0.0		
Cephalosporins	Given	45	83.3	9	16.7	54	P = 0.578
	Not given	6	100.0	0	0.0		
Glucopeptides	Given	14	87.5	2	12.5	16	P = 1.00
	Not given	37	84.1	7	15.9		
Mucositis ⁴	Present	13	81.2	3	18.8	16	P = 1.00
	Not present	21	80.0	4	20.0		
Neutrophil count	<1000	31	86.1	5	13.9	36	P = 1.00
	≥1000	20	83.3	4	16.4		
GU/GI surgery ⁵	Yes	20	83.3	4	16.7	24	P = 1.00
	No	31	86.1	5	13.9		
Urinary catheters	Yes	13	81.3	3	18.7	16	P = 0.689
	No	38	86.4	6	13.6		
Diabetic mellitus	Yes	9	90.0	1	10.0	10	P = 1.00
	No	31	83.8	6	16.2		
CVC ⁶	Yes	7	77.8	2	22.2	9	P = 0.471
	No	8	100.0	0	0.0		
Mechanical ventilation ⁶	Yes	3	75.0	1	25.0	4	P = 0.426
	No	2	92.3	1	7.7		

¹The median days of hospital stay (18.5days) was taken as the cutoff; ²ICU admission within one month of positive culture; ³Within 6 months of chemotherapy treatment; ⁴ mucositis among who received chemotherapy; ⁵ Genito-urinary and gastro intestinal surgery within 1 month; ⁶ only in patients with positive blood culture; CVC-central venous catheter

Table 9: Sensitivity/Specificity of disc diffusion and agar dilution methods compared gradient strip method (gold standard)

Method	sensitivity	specificity
Disc diffusion test	77.8%	100%
Agar dilution test	100.0%	100%

Table: 10 Vancomycin and teicoplanin MIC in vancomycin resistant isolates

Source of isolate	No of isolates	Vancomycin MIC µg/ml	Teicoplanin MIC µg/ml
Blood	1	>256	24
	2	>256	24
Urine	1	>256	12
	2	>256	12
	3	>256	24
	4	12	12
	5	6	6
Perineal abscesses	1	6	0.5
	2	>256	12

Discussion

A prospective study¹² in North India identified *E. faecium* as the commonest blood culture isolate while *E. faecalis* predominated in pus and urine samples. Other species isolated in this study were *E. mundtii*, *E. dispar*, *E. durans*, *E. avium*, *E. raffinosus* and *E. gallinarum*. In another study from New Delhi, *E. faecium* (66%) was the most common isolate in blood samples followed by *E. faecalis* (20%).¹³ However, *E. faecalis* (55%) followed by *E. casseiflavus* (24%) and *E. faecium* (12%) were reported from Chandigarh from urinary isolates.¹⁴ Perera et al.¹⁵ at Sri Jayewardenepura General Hospital, Sri Lanka reported that isolation of *E. faecium* was 45% while *E. faecalis* remained the predominant species. In this study *E. faecium* was the predominant species which caused invasive enterococcal infections (55%) among oncology patients. There are no local or national surveillance data on enterococcal infections in Sri Lanka for comparison.

Most risk factors for VRE infections including prolonged hospital stay and previous antibiotic therapy with multiple antibiotics⁷ are common in oncology patients. According to the antibiotic policy at NCISL since mid-2006, ceftazidime or carbapenems are used as empirical antibiotic therapy in febrile neutropenic patients, depending on the absolute neutrophil count of the patient, after initiating a septic screen. If the patient is hypotensive and in septic shock, a glycopeptide is added after consideration of renal function. Records maintained by the hospital pharmacist during the year 2012 show that NCISL used 69.6 kg of ceftazidime, 3.2 kg of cefotaxime, 4.6 kg of vancomycin, 0.5 kg of teicoplanin 13.2 kg of imipenem and 11.2 kg of meropenem (personal communication). The selective pressure of these empirical antibiotics may be a predisposing factor

for *E. faecium* to become the predominant species and there is a need to conduct a proper case control study to identify the risk factors for VRE.

All the VRE infections were hospital acquired in this study population. However, the study was not designed to elicit an association between the duration of hospital stay and getting an infection with VRE.

The first vancomycin resistant enterococci (VRE) isolate that harboured the *vanA* transposon was identified in 1988 by Uttley *et al*¹⁶ which was reported 30 years after vancomycin was clinically introduced. In 2003, 28.5% of enterococcal strains in the United States were vancomycin resistant.¹⁷ In Australia, in 2005, VRE was low at 0.73%.¹⁸ In 2007, 8.5% of enterococcal isolates in the United Kingdom were found to be vancomycin resistant.¹⁹ Those reports included all enterococci isolates from all patients. In this study the incidence of vancomycin resistant enterococci causing invasive infections in oncology patients was 15%.

In a study evaluating the accuracy of eight currently available test methods (agar dilution, disk diffusion, Etest, agar screen plate, Vitek GPC-TA and GPS-101, and MicroScan overnight and rapid panels) it was shown that *VanA* VRE were detected by all methods but *vanB* VRE were often not detected by Vitek GPS-TA and MicroScan rapid (sensitivities 47% and 53% respectively²⁰ All methods except Etest and agar screen continue to show problems in the detection of *VanC1* and *Vanc2* VRE.²⁰ The sensitivity of the disc diffusion test in detecting *VanA* resistant was 100% but was lower in detection of *VanB*, *VanC1* and *vanC2* (93%,52% and 63% respectively).²⁰

MIC detection by the broth dilution method is considered the gold standard to determine antibiotic sensitivity/resistance according to CLSI guidelines 2013.¹⁰ Due to practical difficulties in using the broth dilution method, the gradient vancomycin strip was used as the standard in this study. The detection of vancomycin resistance by disc diffusion test remained lower (77.8%) than the agar screening test (100%).

MICs of teicoplanin in vancomycin resistant isolates varied from 6-24 µg/ml making these isolates of intermediate sensitivity to teicoplanin. Of 8 vancomycin resistant *E. faecium* isolates 7 isolates were phenotypically compatible with VanA phenotype of glycopeptide resistant enterococci.

Of the 2 *E. casseliflavus* isolates, one was vancomycin susceptible according to the CLSI 2013 criteria. Since *E. casseliflavus* isolates are intrinsically resistant to vancomycin, it is not recommended to treat infections with vancomycin even though the organism shows MICs in the sensitive range.¹⁰ It is therefore important to speciate enterococcal species to detect such anomalies to prevent treatment failures.

Limitations

With this small sample size, precision of findings was low and power to detect the associations between risk factors and vancomycin sensitivity is low. A case control study is better to identify the risk factors.

Conclusions and recommendations

E. faecium was the dominant species (55%) causing invasive enterococcal infections in oncology patients at the National Cancer Institute of Sri Lanka in the study period (2012/13). 78% of vancomycin resistant *E. faecium* in the study were VanA phenotype of glycopeptide resistant enterococci. It is important to speciate enterococci to identify the intrinsically resistant *Enterococci spp* to prevent treatment failures by using inappropriate antibiotics.

Almost all the participants (96.6%) had acquired the enterococcal infection from the hospital. As most risk factors for VRE infections are common in oncology patients, it is recommended that surveillance of VRE is continued with inclusion of a case control study to identify risk factors.

The disc diffusion test was shown to have lower sensitivity in comparison with the agar dilution screening and E test. We therefore recommend one of the latter 2 tests in clinical laboratories investigating patients with invasive enterococcal infections.

Conflict of interest: The authors declare that there are no competing interests.

Ethics statement: Approval from this study was obtained from the Sri Lanka Medical Association ERC/12-016.

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