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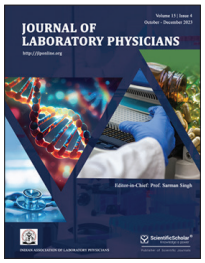

Original Article

Emerging trend of vancomycin-resistant enterococcal bacteremia in a university hospital in Northern India – An observational study

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ABSTRACT

Objectives: Vancomycin Resistant Enterococcus (VRE) species has been increasing and is now of serious concern. The aim of the study was to find the prevalence of VRE bacteremia at our institution. Also, the demographic pattern, associated risk factors, sensitivity pattern and outcomes associated with bacteremia caused by VRE were also estimated.

Materials and Methods: This observational study was done in the Microbiology department of our institute from April 2022 to June 2023. All patients with blood cultures positive for *Enterococcus* species for the first time were included in the study. Identification was done using MALDI-Tof MS. Antimicrobial Sensitivity Testing was and interpreted using Clinical and Laboratory Standards Institute (CLSI) 2023 M-100. The demographic details, risk factors, and the clinical outcome of the patients were collected and analyzed.

Statistical analysis: All the data were entered in Excel sheets. The univariate analysis was done for the risk factors and outcome of the patients with VRE bacteremia and VSE (Vancomycin Sensitive *Enterococcus*) bacteremia. 95% confidence interval, Odd's ratio and p-value was estimated.

Results: During the study period, 29086 blood culture bottles were received. Of these, 2016 (6.93%) bottles flagged positive. Enterococcus species were isolated from 256 (12.69%) blood cultures. Of the 256 Enterococcus isolates, 45 (17.57%) isolates were Vancomycin resistant. Most common species were *Enterococcus faecium* (n=42; 93.33%). Most of the patients belonged to the age group 41-60 years (31.11%). Diabetes mellitus and neutropenia were found to be significant risk factors. All the isolates showed 100% resistance to Teicoplanin, Ampicillin, Ampicillin-sulbactam and Levofloxacin. Mortality was significantly higher in patients with VRE bacteremia as compared to patients with Vancomycin Sensitive Enterococcal (VSE) bacteremia.

Conclusion: Proper implementation of antimicrobial stewardship rules in the hospital is the best way to overcome the increasing trend of resistance.

Keywords: Bacteremia, Diabetes mellitus, Infection, Sepsis, Vancomycin-resistant bacteremia

INTRODUCTION

Antimicrobial resistance is supposed to be the most threatening problem in today's world. Around 1.27 million deaths have been linked to infections caused by bacteria that are antibiotic-resistant. Among these, *Enterococcus faecalis* and *Enterococcus faecium* contribute to around 7.87% and 19.68% of the deaths, respectively.[1] *Enterococcus* species has great potential to attain antimicrobial resistance. Vancomycin-resistant enterococcus (VRE) species have been increasing and are now

of serious concern.[2,3] Vancomycin is a glycopeptide, and it works by binding to the D-Ala-D-Ala terminal of cell wall precursors, thereby inhibiting peptidoglycan synthesis.[3,4] VRE was first reported from Europe in 1988.[5] The first case from India was reported in 1999 from New Delhi.[3] Within a short span of time, it has become one of the predominant causes of nosocomial infections (Tripathi). The World Health Organization has categorized VRE as the most notorious bacteria in the "Global Priority List of Antibiotic-Resistant Bacteria."[3] VRE infection causes deterioration of the patient's condition with 65–70% mortality. Among the risk factors, the major ones associated with VRE bacteremia are prolonged hospitalization, prior exposure to antibiotics such as vancomycin, neutropenia, and renal insufficiency.[5]

The prevalence of VRE bacteremia also varies from place to place, depending on the study population, antibiotics administered, and hospital setting. Therefore, timely analysis of VRE bacteremia must be done in every institution to find the pattern of VRE prevalence. Furthermore, there are limited options for treatment for VRE bacteremia.[3] Keeping this in mind, studies have been done to gain knowledge about the epidemiological factors linked to the emergence of VRE and the cause of the worsening of the condition of patients with VRE bacteremia. Better knowledge of these facts can help in formulating a better control measure which would help in lowering infection with VRE.[6] In a systematic review of the prevalence of vancomycin resistance in India, it was seen that from 2000 to 2022, 19 studies were done on VRE with proper identification and testing of antibiotic sensitivity, of which 7 studies [Table 1] were from North India.[7-13] However, among these, only two studies were done on blood samples, which urges the need for more focused studies from North India on VRE bacteremia.[1] Thus, for the above-listed reasons, there was a need to find the prevalence of VRE bacteremia at our institution. Furthermore, the demographic pattern, associated risk factors, sensitivity pattern, and outcomes associated with VRE bacteremia were also estimated.

Highlights

- *Enterococcus* species were isolated from 12.69% blood cultures.
- *Enterococcus faecium* (*n* = 187; 73.04%) was the predominant isolate in the study
- Of the *Enterococcus* isolates, 17.57% isolates were vancomycin-resistant enterococcus.

MATERIALS AND METHODS

This observational study was done in the microbiology department of our institute from April 2022 to June 2023. All patients with blood cultures positive for *Enterococcus* species for the first time during the study period were included in the study.

Case definition

A case of enterococcal bacteremia was defined as the presence of clinical symptoms such as fever, chills, or hypotension, along with microbiological evidence of *Enterococcus* species isolated from blood cultures. Furthermore, the pathogen should not be isolated from any other site of the patient.

Blood culture collection

As and when required, the blood culture samples from patients were sent to the microbiology laboratory by treating clinicians in automated BACT/ALERT aerobic blood culture bottles which were loaded in the automated BACT/ALERT 3D system as soon as they were received. The time to positivity (TTP) was also noted when the bottle flagged a positive signal.

Sample processing

Gram staining was done directly from the positive bottle, followed by culture on 5% sheep blood agar and MacConkey agar, and the culture plates were incubated aerobically at 37°C. The identification of the isolate was done from the colony using the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (matrix-assisted laser desorption ionization–time-of-flight mass spectrometry, VITEK MS, Biomeriuex).

Antibiotic sensitivity testing

For antimicrobial susceptibility, the reference strain used was *E. faecalis* ATCC 29212. Inocula were prepared from the overnight growth on a blood agar plate by suspending seven to eight morphologically similar colonies in nutrient broth. Each inoculum was adjusted to 0.5 McFarland standards. Then, Antimicrobial Sensitivity Testing was performed using by Kirby Bauer disk diffusion method. The susceptibility results were read and interpreted after 16–18 h of incubation using Clinical and Laboratory Standards Institute 2023 M-100 clinical breakpoints. The antibiotics tested were ampicillin (10 μg), ampicillin-sulbactam (10/10 μg), high-level gentamicin (120 μg), doxycycline (30 μg), minocycline (30 μg), levofloxacin (5 μg), vancomycin (30 μg), linezolid (30 μg), and teicoplanin (30 μg).

Data collection

The demographic details, risk factors, and clinical outcomes of the patients with VRE bacteremia were collected from Hospital Information Software and analyzed.

Statistical analysis

All the data were entered in Excel sheets. The univariate analysis was done for the risk factors and outcome of the

Table 1: Publications documented on VRE infection from Northern India from 2000 to 2022.

Authors	Year of publication	Year of study	Study population	Sample	Total number of Enterococcal isolates	Number of *E. faecium* isolates	Number of *E. faecalis* isolates	Total number of VRE isolates	Number of *E. faecalis* isolates among VRE isolates	Number of *E. faecium* isolates among VRE isolates E
Das *et al.*[7]	2022	2016–2018	Patients symptomatic of UTI	Urine	118	94	20	20 (16.95%)	14	6
Goel *et al.*[8]	2016	2013–2014	Patients suspected of UTI	Urine	115	61	42	13 (11.3%)	6	5
Jain *et al.*[9]	2022	2014–2015	Post Caesarean section surgical site infection	Infected surgical swab	9	–	–	3 (33.33%)	–	–
Kapoor *et al.*[10]	2005	2001	Children admitted with bacteremia	Blood	50	10	33	0	0	1
Meena *et al.*[11]	2017	2015–2016	In-patients and out-patients symptomatic of UTI	Urine	70	9	61	26 (37.14%)	1	25
Purohit *et al.*[12]	2017	2013–2015	Hospitalized patients	Blood, Urine, pus	250	82	162	57 (22.8%)	2	55
Taneja *et al.*[13]	2004	2000–2001	Patients suspected of UTI	Urine	144	80	17	8 (5.56%)	1	5

UTI: Urinary tract infection, VRE: Vancomycin-resistant enterococcus, *E. faecium: Enterococcus faecium, E. faecalis: Enterococcus faecalis*

patients with VRE bacteremia and vancomycin-sensitive enterococcus (VSE) bacteremia. About 95% confidence interval, Odd's ratio, and *P*-value were estimated. $P < 0.05$ was considered as statistically significant.

Bias

We have tried to include all the blood culture isolates that were received in our laboratory so that all possible chances of error and bias could be avoided.

Ethical approval

The study design was approved by the Institutional Ethics Committee (IEC code: 2022-109-IMP-EXP-35).

RESULTS

During the study period, 29,086 blood culture bottles were received in the bacteriology laboratory of our department. Of these, 2016 (6.93%) bottles flagged positive. *Enterococcus* species were isolated from 256 (12.69%) blood cultures. The median TTP for an *Enterococcus* isolate was 12.6 hours. Among the 256 patients with positive *Enterococcus* culture, male predominance was seen among the patients (Male: *n* = 148; 57.8%, Female: *n* = 108; 42.18%). Most of the patients were of the age group 41–60 years [Table 2]. *E. faecium* (*n* = 187; 73.04%) was the predominant isolate in the study, followed by *E. faecalis* (*n* = 68; 26.56%). Other species which were isolated were *Enterococcus gallinarum* (*n* = 1; 0.39%) [Table 3].

On performing antibiotic sensitivity testing with vancomycin, it was found that 45 (17.57%) isolates were VRE. The most common species was *E. faecium* (*n* = 42; 93.33%), followed by *E. faecalis* (*n* = 2; 4.44%) and *E. gallinarum* (*n* = 1; 2.22%) [Table 3] Among the 45 patients with VRE, majority of the patients were of the age group 41–60 years (*n* = 14; 31.11%). Male: Female ratio was 1.25:1 [Table 2]. From Table 4, the maximum number of patients were from critical care medicine (*n* = 27; 60%), followed by gastroenterology (*n* = 3; 6.66%) and hepatology (*n* = 3; 6.66%). Most of the patients had a diagnosis of acute kidney injury (*n* = 10; 22.22%) followed by multiple organ dysfunction syndrome (*n* = 7; 15.55%) [Table 5].

Of the 45 patients with VRE bacteremia, some patients had risk factors such as diabetes mellitus (*n* = 25; 55.55%), transplant recipients (*n* = 2; 4.44%), steroid therapy (*n* = 5; 11.11%), carcinoma patients (*n* = 6; 13.33%), patients who underwent surgical procedures in last 30 days (*n* = 2; 4.44%), and patients with neutropenia (*n* = 10; 22.22%). Some patients also had more than one risk factor present. On univariate analysis, it was seen that risk factors such as diabetes mellitus and neutropenia had a significant association with VRE bacteremia [Table 6].

All the isolates showed 100% resistance to teicoplanin, ampicillin, ampicillin-sulbactam, and levofloxacin. None of the isolates showed resistance to linezolid [Table 7].

Table 2: Demographic details of the patients with enterococcal bacteremia. Details of patients with VRE and VSE bacteremia are also listed.

	Total number of patients (*n*=256) (%)	Number of patients with VRE bacteremia (*n*=45) (%)	Number of patients with VSE bacteremia (*n*=211)
Gender			
Male	148 (57.8)	25 (55.55)	123 (58.29)
Female	108 (42.18)	20 (44.44)	88 (41.7)
Age group			
0–20 years	43 (16.79)	7 (15.55)	35 (16.58)
21–40 years	61 (23.82)	11 (24.44)	49 (23.22)
41–60 years	80 (31.25)	14 (31.11)	67 (31.75)
61–80 years	60 (23.43)	13 (28.88)	48 (22.74)
81–100 years	12 (4.68)	–	12 (5.68)

VRE: Vancomycin-resistant enterococcus, VSE: Vancomycin-sensitive enterococcus

Table 3: Species distribution of the isolates in the study.

Species isolated	Total isolates (*n*=256) (%)	Number of patients with VRE bacteremia (*n*=45) (%)	Number of patients with VSE bacteremia (*n*=211) (%)
Enterococcus faecalis	68 (26.56)	2 (4.44)	66 (31.27)
Enterococcus faecium	187 (73.04)	42 (93.33)	145 (68.72)
Enterococcus gallinarum	1 (0.39)	1 (2.22)	-

VRE: Vancomycin-resistant enterococcus, VSE: Vancomycin-sensitive enterococcus

Table 4: Distribution of patients of VRE bacteremia according to the wards where they were admitted.

Wards	Number of patients with VRE bacteremia (*n*=45) (%)
Cardiology	1 (2.22)
Critical Care Medicine	27 (60)
Emergency	1 (2.22)
Gastroenterology	3 (6.66)
Gastrosurgery	1 (2.22)
Hematology	1 (2.22)
Hepatology	3 (6.66)
Immunology	1 (2.22)
Nephrology	1 (2.22)
Neurosurgery	1 (2.22)
Neonatal ICU	1 (2.22)
Pediatric gastroenterology	1 (2.22)
Trauma center	1 (2.22)
Urology	2 (4.44)

ICU: Intensive care unit, VRE: Vancomycin-resistant enterococcus

Table 5: Diagnosis of the patients with VRE bacteremia.

Diagnosis	Number of patients with VRE bacteremia (*n*=45) (%)
Acquired TEF	1 (2.22)
Acute decompensated heart failure	1 (2.22)
Acute leukemia	1 (2.22)
Acute necrotizing pancreatitis	1 (2.22)
Acute on chronic cholecystitis	1 (2.22)
ALL	1 (2.22)
Anti-NMDA antibody receptor positive autoimmune encephalitis	1 (2.22)
Bilateral hydroureteronephrosis with meningitis	1 (2.22)
B-lymphoblastic lymphoma	1 (2.22)
Acute kidney injury	10 (22.22)
Carcinoma head of pancreas	1 (2.22)
Choledocholithiasis	2 (4.44)
Chronic liver disease	2 (4.44
Chronic kidney disease	4 (8.88)
MODS	7 (15.55)
Liver abscess	3 (6.66)
Hepatitis encephalopathy	1 (2.22)
SLE	3 (6.66)
Emphysematous polynephritis	1 (2.22)
Biliary stricture	2 (4.44)

VRE: Vancomycin-resistant enterococcus, TEF: Tracheoesophageal fistula, SLE: Systemic lupus erythematosus, NMDA: N-methyl D-aspartate, MODS: Multiple organ dysfunction syndrome, ALL: Acute lymphocytic leukemia

Among the patients with VRE bacteremia (n = 45), 16 (35.55%) patients were completely cured and were discharged in stable condition. Mortality was attributable to bacteremia in 29 (64.44%) patients [Table 6]. Among the 29 patients, sepsis worsened, and multiorgan dysfunction syndrome developed in 7 (24.13%) patients. Diabetes mellitus was a risk factor in 12 (41.37%) of the patients who died. On univariate analysis, mortality was significantly higher in patients with VRE bacteremia as compared to patients with VSE bacteremia [Table 6].

DISCUSSION

Enterococcal bacteremia is of great concern not only due to the increasing prevalence but also due to the increasing resistance pattern.[3,5] It is one of the main causes of nosocomial infection. Vancomycin has been known to be a better option for the treatment of serious infection in critically ill patients admitted to Intensive Care Units (ICUs).[14] Besides that, it is capable of transferring its vancomycin-resistant genes (*van* genes) to methicillin-resistant *Staphylococcus aureus* by means of gene transfer, which increases the threat. The most common cause of increasing vancomycin resistance is the overuse of cell wall-acting antibiotics, causing selection pressure.[3] Phenotypically, eight variants of glycopeptide resistance have been documented. They are VanA, VanB, VanC, VanD, VanE, VanG, VanL, VanM, and VanN. Of these, VanC is the factor responsible for intrinsic resistance, and all other phenotypes lead to acquired resistance. Intrinsic resistance due to VanC is seen in *E. gallinarum* and *Enterococcus casseliflavus*.[4]

The prevalence of VRE bacteremia was found to be 17.57% in the present study. A study from our institution in 2006 documented a VRE bacteremia prevalence of only 1.4%.[15] This implies that there has been a rising trend of vancomycin resistance among *Enterococcus* species. Other studies have shown a prevalence of 14.7%,[3] 7.9%,[5] 25.2%,[12] 6.1%,[16] 14.09%,[17] and 19.6%.[18] A study from Germany documented a rise in trend of VRE bacteremia from 5% in 2001 to 14.5% in 2013.[19]

The most common age group affected with VRE bacteremia was 41–60 years, followed by 61–80 years [Table 2]. This implies that the elderly population is more affected by vancomycin-resistant enterococcal bacteremia, and this might be due to the weakened immune system. This finding is supported by other studies also. In a study by Tripathi *et al*., the median age of patients with VRE bacteremia was 54.69 years.[5] Furthermore, in a study by Sivaradjy *et al*., the mean age group of the patients of VRE bacteremia was 56 years.[3] Diabetes mellitus and neutropenia were among the significant risk factors for VRE bacteremia in this study. Diabetes was documented as a significant risk factor in a study by Tripathi *et al*., also.[5]

Table 6: Univariate analysis of the risk factors and the outcomes of the patients with VRE and VSE bacteremia.

	Number of patients with VRE bacteremia (*n*=45) (%)	Number of patients with VSE bacteremia (*n*=211) (%)	95% CI	Odd's ratio	*P*-value
Risk factors/comorbidities					
Diabetes mellitus	25 (55.55)	46 (21.80)	2.28–8.78	4.489	<0.0001
Transplant recipients	2 (4.44)	10 (4.73)	0.19–4.42	0.939	0.932
Steroid therapy	5 (11.11)	13 (6.16)	0.64–5.63	1.903	0.245
Carcinoma patients	6 (13.33)	21 (9.95)	0.52–3.67	1.391	0.504
Surgical procedure in the past 30 days	2 (4.44)	12 (5.68)	0.16–3.57	0.771	0.739
Neutropenia	10 (22.22)	23 (10.9)	1.02–5.33	2.33	0.04
Outcome					
Dead	29 (64.44)	26	6.18–26.91	12.89	<0.0001
Recovered	16 (35.55)	185			

VRE: Vancomycin-resistant enterococcus, VSE: Vancomycin-sensitive enterococcus, CI: Confidence interval

Table 7: List of antibiotics tested in VRE isolates and the number of resistant isolates.

Antibiotics tested	Isolates of VRE showing resistance to the antibiotics tested (*n*=45) (%)
Teicoplanin	45 (100)
High level gentamicin	42 (93.33)
Ampicillin	45 (100)
Ampicillin-sulbactam	45 (100)
Doxycycline	20 (44.44)
Levofloxacin	45 (100)
Linezolid	0
Minocycline	1 (2.22)

VRE: Vancomycin-resistant enterococcus

As seen in the present study, patients admitted to ICU were most affected by VRE bacteremia. This was seen in studies by Tripathi *et al.* (34.7%)[5] and Sivaradjy *et al.* (68%).[3] The reason for this increased nosocomial prevalence of VRE bacteremia could be due to patient risk factors like underlying illness, extremes of age, or presence of invasive medical devices in ICUs like intravascular catheters or due to fomite reservoirs, which contribute to cross infections in ICUs.[5]

Among the *Enterococcus* species, *E. faecium* ($n = 42$; 93.33%) is mainly responsible for VRE bacteremia, followed by *E. faecalis* ($n = 2$; 4.44%). This has been supported by studies by Tripathi *et al.*, (*E. faecium*: $n = 46$; 10.9%, *E. faecalis*; $n = 72$; 6.7%).[5]

Variable sensitivity pattern was seen in other studies. In a study by Sivaradjy *et al.*, reduced susceptibility was seen for levofloxacin, ampicillin, and teicoplanin.[3] All these antibiotics showed 100% resistance in the present study. Linezolid was sensitive in all isolates in this study. In a study By Tripathi *et al.*, also, 100% sensitivity was also seen with linezolid.[5] This was in discordance with a study by Sivaradjy *et al.*, where they reported an emerging trend of linezolid resistance among Enterococcus species, with 12.7% of isolates showing resistance.[3]

Mortality due to VRE bacteremia was significantly high (57.78%) in this study. This was higher as compared to other studies in the literature. The mortality rates due to VRE bacteremia, as documented by other studies, were 37% in 1999,[20] 21.3% in 2000,[21] 15.3% in 2009,[16] and 22.8% in 2016.[5] This might be due to the fact that our center is a tertiary care referral center and since most of the patients admitted to our institute are referred from other settings. Hence, the infection prevalence rates are higher in our institution as compared to other settings. Mortality has been seen to be significantly higher in patients with VRE bacteremia as compared to VSE bacteremia in this study. O'Driscoll and Crank have also documented that mortality rates are 2.5 times higher among patients with VRE bacteremia as compared to VSE bacteremia.[4]

CONCLUSIONS

An increasing prevalence of VRE bacteremia was observed in our study compared to the previous literature. This is of great concern as this is mainly due to overuse and misuse of antibiotics. Proper implementation of antimicrobial stewardship rules in the hospital is the best way to overcome the increasing trend of resistance.

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Ethical approval: The study was approved by the Institutional Ethics Committee with approval number (IEC code: 2022-109-IMP-EXP-35), dated 16th March 2022.

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