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# Risk factors for intestinal colonization with vancomycin resistant enterococci' A prospective study in a level III pediatric intensive care unit

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## Abstract:

**PURPOSE:** Vancomycin-resistant enterococci (VRE) emerged as one of the major nosocomial pathogens across the globe. Gut colonization rate with VRE is higher in patients admitted to intensive care units (ICUs) due to the higher antibiotic pressure. VRE colonization increases the risk of developing infection up to 5–10 folds. The aim of this study was to determine the rates of VRE colonization among the patients admitted to pediatric ICU (PICU) and risk factors associated with it.

**MATERIALS AND METHODS:** Rectal swabs were collected after 48 h of admission to PICU from 198 patients. The samples were inoculated onto bile esculin sodium azide agar with 6 mg/ml of vancomycin. Growth on this medium was identified by the standard biochemical test, and minimum inhibitory concentration of vancomycin and teicoplanin was detected by agar dilution method. Resistance genes for vancomycin were detected by polymerase chain reaction. Risk factors were assessed by logistic regression analysis.

**RESULTS:** The rates of VRE colonization in patients admitted to PICU was 18.6%. The majority of the isolates were *Enterococcus faecium* (75.6%) followed by *Enterococcus faecalis* (24.4%). One patient acquired a VRE bloodstream infection (2.6%) among colonized patients, and none of the noncolonized patients acquired the infection. Consumption of vancomycin was found to be the only risk factor significantly associated with VRE colonization.

**CONCLUSION:** Routine surveillance and isolation of patients found to be VRE colonized may not be possible in tertiary care hospitals; however, educating health-care workers, promoting handwashing with antiseptic soaps or solutions, and antibiotic Stewardship policy may help in the reduction of vancomycin resistance and VRE colonization.

## Keywords:

*Enterococcus faecalis*, *Enterococcus faecium*, vancomycin-resistant enterococci, vancomycin-resistant enterococci, vancomycin-resistant enterococci colonization in Intensive Care Unit

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## Introduction

After the first report of vancomycin-resistant enterococci (VRE) was published from the UK and France in 1986, it has emerged as one of the major nosocomial pathogens across the globe.<sup>[1]</sup> Long-term stay in the hospitals may result in VRE colonization in the gut thus creating reservoirs of infection.

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Transmission of VRE from patient to patient occurs through hands of health-care workers and through contaminated fomites in the environment.<sup>[2]</sup> VRE colonization increases the risk of developing infection up to 5–10 fold.<sup>[3]</sup>

*Enterococcus faecalis* and *Enterococcus faecium* contribute to 80%–90% of all enterococcal infections such as bloodstream infection,

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endocarditis, meningitis, urinary tract infection, and wound infection.<sup>[4]</sup> Multidrug resistance, increased rate of gut colonization, use of broad-spectrum antibiotics, and invasive devices have been implicated in the emergence of VRE.<sup>[5]</sup> VRE bacteremia usually results in increased mortality rate and treatment cost when compared to bacteremia caused by vancomycin-sensitive enterococci.<sup>[6,7]</sup>

VRE colonization is a major burden in intensive care units (ICU's) of large tertiary care hospitals. The higher antibiotic pressure in the ICU's contributes to the increased rate of VRE colonization. Once patients get colonized with VRE, it may persist from 7 weeks to 3 years, which may lead to a variety of infections in ICU patients.<sup>[8]</sup> The risk factors, namely, prolonged hospitalization, immunosuppression, low birth weight, younger age group, and prolonged antibiotic consumption of vancomycin, teicoplanin, amikacin, and ceftazidime were found to be associated with VRE colonization in pediatric and newborn ICUs and also in children's hospitals.<sup>[5,9-15]</sup>

There are limited reports of VRE colonization particularly from pediatric ICU (PICU) across the globe with rates ranging from 0% to 66%.<sup>[2,5,9,16-23]</sup> A study from our neighboring country Pakistan (Southeast Asia) reported a VRE colonization in PICU of 10.7%.<sup>[5]</sup> At the time of undertaking this study, to the best of our knowledge, no reports were available from India on VRE colonization in PICU's. The main objectives of this study were to determine the rate of VRE colonization in patients admitted to PICU and to assess the risk factors associated with it.

## Materials and Methods

### Study design and subject

A prospective study was conducted in level III PICU of a tertiary care teaching hospital from September 2013 to February 2015. Written informed consent was obtained from parents/guardians of the admitted patients, and the study was approved by the Institutional Ethics Committee.

### Intervention/data collection

A structured pro forma was used to document the patient's demographic and clinical details, which included age, sex, medical history, clinical diagnosis, prior hospital/ICU admission, date of present admission to hospital and PICU, and history of antibiotic usage.

### Outcome measures

- Screening rates for VRE in patients admitted to PICU
- Risk factors associated with VRE colonization.

### Sample size

The sample size was calculated to be 196 using open Epi Version 3.01 (Armonk, NY, IBM Corp), taking the 17% positivity rate from a pilot study with 5% absolute precision, 5% alpha error, and 10% nongrowth or contaminated samples.

### Inclusion criteria

The patients who were admitted for >48 h in the ICU with the consent from parents/guardians were included in the study.

### Screening and diagnostic procedures

During the study period, 1290 patients were admitted in the PICU, out of which 1018 patients stayed >48 h. As calculated sample size was 196, convenient sampling was carried out to achieve the sample size. Rectal swabs were collected after 48 h of admission to PICU and inoculated onto bile esculin sodium azide agar containing 6mg/L vancomycin (Himedia laboratories, Mumbai, India).<sup>[5]</sup> Black/brown colonies were presumptively identified as *Enterococcus* and confirmed to species level based on Facklam and Collins standard biochemical tests.<sup>[24]</sup> Minimum inhibitory concentration (MIC) of vancomycin and teicoplanin was determined by agar dilution method for all the enterococcal isolates grown on BEA as per the Clinical and Laboratory Standard Institute guidelines.<sup>[25]</sup> MIC of  $\geq 32$  was considered as resistant to both vancomycin and teicoplanin. The antimicrobial susceptibility of the isolates to other antibiotics, namely, ampicillin, high-level gentamicin, tetracycline, and linezolid was also performed by Kirby-Bauer disc diffusion method.<sup>[26]</sup> Association of VRE colonization with demographic features of the patients and various risk factors for VRE colonization were assessed based on previous studies.

### Molecular detection of vancomycin resistance genes

Genomic DNA was extracted from all the phenotypically vancomycin-resistant isolates using Qiagen mericon DNA extraction kit as per the manufacturer instructions. Polymerase chain reaction (PCR) was carried out to detect the vancomycin-resistance genes, namely, *vanA*, *vanB*, *vanC-1*, *vanC-2*, and *vanC-3*, using published primers.<sup>[27]</sup> PCR was done in a Mastercycler nexus (Eppendorf) with initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, annealing temperature at 54°C for 1 min, and extension at 72°C for 1 min, followed by the final extension at 72°C for 10 min. PCR products were run on 1.5% agarose gel with 100 bp ladder, stained with ethidium bromide and visualized under Gel Doc XR + System (Bio-Rad Laboratories).

### Statistical analysis

Data were analyzed using SPSS 20.0 version software (OpenEpi: Open Source Epidemiologic Statistics for

Public Health, Version. www.OpenEpi.com). The outcome of colonization with VRE was expressed as a binary categorical variable. All categorical variables were analyzed using Chi-square test while logistic regression was used for continuous independent variables and categorical outcome. The  $P < 0.05$  was considered statistically significant. The difference in difference test was used to analyze the difference between the proportions of VRE-positive cases in PICU.

## Results

Out of 1290 patients admitted in PICU during the study period, 198 were screened for VRE. The detailed flow chart is mentioned in Figure 1.

The rectal swabs were collected from 198 patients admitted to PICU, out of which 37 (18.6%) yielded VRE. Of the VRE isolates, 75.6% were *E. faecium* and 24.4% were *E. faecalis*. The MIC values for both vancomycin and teicoplanin ranged from 64 to  $\geq 256$  for all the VRE isolates. Vancomycin resistance was always associated with teicoplanin resistance indicating that these VRE isolates were vanA-phenotype. PCR also confirmed *vanA* gene in all the 37 VRE isolates and none of them were positive for *vanB* and *vanC* genes. Very high rates of resistance to other antibiotics were noted with disc diffusion method (89%, 82%, and 86% to ampicillin, high-level gentamicin, and tetracycline, respectively). No resistance was observed to linezolid.

Among the various risk factors assessed, none of the demographic features of the patients admitted to PICU were associated with VRE Colonization [Table 1]. The average patient stay in the PICU before sample collection was 3.5 days (standard deviation  $\pm 3.84$ ). No statistically significant association was found with VRE colonization rate and duration of patient stay, history of (H/O)

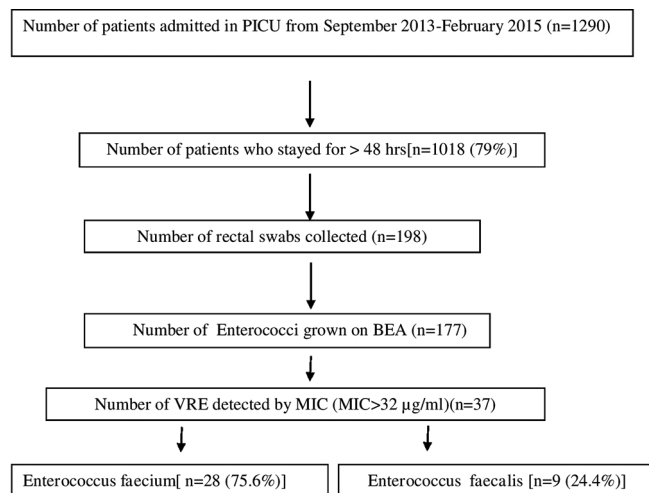


Figure 1: Flow chart of study

previous hospital stay, and H/O surgery or urinary catheterization in PICU. Among the various antibiotics, vancomycin consumption was the only risk factor found to be significantly associated with VRE colonization in patients admitted to PICU ( $P = 0.006$ ) [Table 2]. The average duration of vancomycin consumption was 2.2 days among VRE colonizers and 1.9 days among noncolonizers before sample collection. Of the 37 colonized patients, one patient acquired bloodstream infection after 7 days of VRE colonization. On the other hand, none of the 161 noncolonized patients acquired VRE infection.

## Discussion

This study was conducted to detect the rates of VRE colonization among the patients admitted to PICU in a larger tertiary care hospital in southern India. ICUs are the major pool of infections with amplifying antimicrobial resistance. The heavy antibiotic burden in the ICUs provide a favorable environment for the development of resistance.<sup>[28]</sup> In this study, the rate of gut colonization with VRE was found to be 18.6% among patients admitted to PICU. In a study by Yameen *et al.*, 2013, from Pakistan, 10.7% of VRE colonization rate was reported from patients admitted to PICU.<sup>[5]</sup> Nateghian *et al.*, 2014,<sup>[23]</sup> from Iran, reported a high incidence of VRE colonization (66%). The rate of VRE colonization (18.6%) in our study correlates with other studies conducted in pediatric hospitals.<sup>[3,20-22]</sup> However, these studies also included patients admitted to pediatric wards in addition to ICUs. A few studies that include NICU patients reported higher rates of VRE colonization ranging from 39% to 66%.<sup>[2,9,23]</sup> whereas in a study reported by Bakir Saygan *et al.*, 2010<sup>[18]</sup> and Singh *et al.*, 2005,<sup>[19]</sup> from NICU, lower rates of VRE colonization of 0% and 3.5%, respectively, were encountered.

A meta-analysis study comprising adult patients admitted to hospitals in the USA reported an average VRE colonization rate of 12.5% with a range of 0%–42%.<sup>[29]</sup> However, data obtained from all the ICU's, that is, neonatal, pediatrics, and adults, VRE colonization rate varies from 0% to 66%.<sup>[21-23,27-29]</sup> By comparing all these studies, it is clear that there is no specific association between age of the patients and colonization rates. These dissimilarities in the colonization rates may be dependent on geographical variation, hospital infection control policies, differences in health-care personal practices, and methodologies followed for the detection of colonization.

Previous studies have reported the association of various risk factors for VRE colonization such as prolonged hospitalization, immunosuppression, younger age group, prolonged antibiotic consumption such as vancomycin, teicoplanin, amikacin, and ceftazidime, administration

of antimicrobial therapy for the late onset of neonatal sepsis.<sup>[1,5,9-15]</sup> In this study, the consumption of vancomycin was the only risk factor significantly associated with the VRE colonization. In contrast, in a study from our center in adult patients admitted to MICU, we observed prior ceftriaxone administration to be a significant risk factor.<sup>[30]</sup>

All the VRE isolates in this study had higher MIC value ranging from 64  $\geq$  256 mg/L to both vancomycin and teicoplanin. This result depicts that all the strains are vanA-phenotype, that is, high-level resistance to both vancomycin and teicoplanin. Although the VRE isolates

from this study showed very high rates of resistance to other antibiotics (89%, 82%, and 86% to ampicillin, high-level gentamicin, and tetracycline, respectively), no resistance was observed against linezolid. A few studies reported the emergence of resistance to linezolid among VRE isolates from colonized patients.<sup>[31]</sup> Antibiotics such as linezolid, tigecycline, and daptomycin are the treatment of choice for VRE infections, and most of the studies showed 100% sensitivity to these higher antibiotics.<sup>[32]</sup>

van A and van B are more commonly found among VRE isolates followed by vanC.<sup>[33]</sup> We also looked for the presence of *van* genes, that is, *vanA*, *vanB*, *vanC-1*, *vanC-2*, and *vanC-3*. In our study, only *vanA* gene was detected in all the VRE isolates, and no *vanB* and *vanC* genes were detected. vanA phenotypes are resistant to both vancomycin and teicoplanin due to the presence of *vanA* gene whereas vanB phenotype is only resistant vancomycin and sensitive to teicoplanin and carry *vanB* genotype. There is a variation in the global distribution of the van phenotype and genotypes. The majority of the VRE reported from the USA and Israel is the vanA phenotype while in Australia vanB phenotype is predominant.<sup>[34]</sup> In an earlier study from our hospital, we had reported both vanA and vanB phenotypes isolated from various clinical

**Table 1: Association of demographic features with vancomycin-resistant enterococci colonization among patients admitted to pediatric intensive care unit (n=198) (logistic regression analysis)**

Variables	Patients colonized with VRE, (n=37) n (%)	Patients not colonized with VRE, (n=161) n (%)	OR (95%-CI)	P
Age in years, mean $\pm$ SD	2.59 (2.18)	2.44 (1.77)	1.16 (0.95-1.30)	0.17
Female	19 (51.4)	83 (51.6)	0.99 (0.49-2.03)	0.98
Male	18 (48.6)	78 (48.4)	1	

VRE = Vancomycin-resistant enterococci, SD = Standard deviation, OR = Odds ratio, CI = Confidence interval

**Table 2: Association of risk factors with vancomycin-resistant enterococci colonization among patients admitted to pediatric intensive care unit (n=198) (logistic regression analysis)**

Variables	Patients colonized with VRE, (n=37) n (%)	Patients not colonized with VRE, (n=161) n (%)	OR (95%-CI)	P
Urinary catheterization	23 (62.2)	108 (66.2)	1.081 (0.38-1.69)	0.56
No urinary catheterization	14 (37.8)	53 (32.9)	1	
History of previous hospitalization	3 (8.1)	9 (5.6)	1.49 (0.38-5.80)	0.56
No history of previous hospitalization	34 (91.9)	152 (94.4)	1	
Duration of current hospital stay before specimen collection, mean $\pm$ SD	3.84 (1.8)	3.47 (1.6)	1.27	0.25
History of surgery	3 (8.1)	17 (10.6)	0.75 (0.21-2.70)	0.65
No history of surgery	34 (91.9)	144 (89.4)	1	
Vancomycin given	23 (62.2)	28 (17.4)	7.80 (3.58-17.01)	0.00
Vancomycin not given	14 (37.8)	133 (82.6)	1	
Ceftriaxone given	16 (43.2)	70 (43.5)	0.99 (0.48-2.04)	0.97
Ceftriaxone not given	21 (56.8)	91 (56.5)	1	
Metronidazole given	14 (37.8)	49 (30.4)	1.39 (0.66-2.93)	0.38
Metronidazole not given	23 (62.2)	112 (69.6)	1	
Meropenem given	7 (18.9)	34 (21.1)	0.87 (0.35-2.16)	0.76
Meropenem not given	30 (81.1)	127 (78.9)	1	
Cefoperazone-sulbactam given	5 (13.5)	29 (18)	0.71 (0.26-1.98)	0.51
Cefoperazone-sulbactam not given	32 (86.5)	132 (82)	1	
Azithromycin given	20 (54.1)	80 (49.7)	1.19 (0.58-2.44)	0.63
Azithromycin not given	17 (45.9)	81 (50.3)	1	
Amikacin given	15 (40.5)	61 (37.9)	1.12 (0.54-2.32)	0.76
Amikacin not given	22 (59.5)	100 (62.1)	1	
Levofloxacin given	3 (8.1)	21 (13)	0.59 (0.17-2.09)	0.40
Levofloxacin not given	34 (91.9)	140 (87)	1	
Gentamicin given	15 (40.5)	51 (31.7)	1.47 (0.70-3.07)	0.30
Gentamicin not given	22 (59.5)	110 (68.3)	1	

VRE = Vancomycin-resistant enterococci, SD = Standard deviation, OR = Odds ratio, CI = Confidence interval



samples although in that study VRE isolates from both ICU and non-ICU patients were included.<sup>[4]</sup>

Out of 37 colonized patients in PICU, one patient (2.6%) acquired a VRE bloodstream infection after 7 days of detection of VRE colonization, while none of the 161 noncolonized patients was infected. VRE bloodstream infection rate among colonized patients ranging from 0% to 45% have been reported in many other studies.<sup>[3]</sup> The VRE isolated from rectal swab and blood of the same patient was of the same species, that is, *E. faecium* and exhibited similar antimicrobial sensitivity pattern. Based on these findings, we came to the purely tentative conclusion that the same strain went on to cause the infection. However, molecular characterization such as pulsed-field gel electrophoresis and multilocus sequence typing would have given a clearer picture about clonal relatedness.

The increase in VRE colonization and infection may lead to the risk of developing vancomycin resistance in *Staphylococcus aureus* strains. The colonization of patients with VRE and methicillin-resistant *S. aureus* (MRSA) increases the risk of acquiring vancomycin resistance by MRSA through plasmid-mediated gene transfer.<sup>[34]</sup> Hospital infection control practices advisory committee published the first guidelines in 1994 for the control of VRE infection in the hospitals.<sup>[35]</sup>

Limitation of the study is that rectal swab was collected at a single point of time. Follow-up rectal swabs of initially noncolonized patients would have provided information on VRE acquisition rate during the stay. We did not include pediatric control group in non-ICU areas which could have revealed any differences in the rates of VRE colonization in different hospital areas.

## Conclusion

VRE colonization rate is moderately high in PICU of our hospital with prior vancomycin usage the only significant risk factor. Routine surveillance and isolation of patients found to be VRE-colonized may not be feasible in tertiary care hospitals; however, educating health-care workers, promoting handwashing with antiseptic soaps or solutions, and antibiotic Stewardship policy may help in the reduction of vancomycin resistance and VRE colonization.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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