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Fecal Carriage of VRE in Intensive Care Unit Patients: A Cross-Sectional Study

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Abstract: *Background:* VRE have emerged as significant nosocomial pathogens, with gastrointestinal colonization serving as a potential source for invasive infections, particularly in critically ill patients. *Objective:* This cross-sectional study was conducted to determine the fecal carriage rate of VRE among patients admitted in surgical ICU for >48 hrs. *Methods:* Rectal swabs were collected and processed in MacConkey agar. Enterococci were identified using colony morphology and conventional identification tests. VRE screening was performed using Vancomycin screen agar (6 µg/mL). *Results:* Among 80 patients screened, 7 (8.75%) tested positive for VRE. Risk factors identified among colonized individuals included prolonged hospital stay, abdominal surgery, and prior use of antibiotics such as Piperacillin-tazobactam, Metronidazole, Ceftriaxone, and Amikacin. *Conclusion:* The study highlights a moderate prevalence of VRE colonization in the surgical ICU setting and emphasizes the need for routine screening, risk-based surveillance, and strict infection control practices to prevent the spread of VRE within healthcare facilities.

Keywords: Vancomycin Resistant Enterococci (VRE), ICU, Fecal Carriage, Infection Control Practices.

Introduction

Enterococcus species are the normal microbiota of the human gastrointestinal tract, oral cavity, and, to a lesser extent, the genitourinary tract. Despite being natural colonizers, they have emerged as significant opportunistic pathogens, causing wound infections, endocarditis, urinary tract infections and bacteraemia, particularly in hospitalized and immunocompromised individuals. Among the various species, *Enterococcus faecalis* and *Enterococcus faecium* are the most frequently isolated in clinical settings [1-2].

Colonization of the gastrointestinal tract with Vancomycin Resistant *Enterococcus* (VRE) is often asymptomatic but represents a significant risk factor for subsequent invasive infections, particularly in vulnerable populations such as the Intensive Care Unit (ICU) patients. Colonization serves as a reservoir from which systemic infections can arise, particularly under conditions of mucosal barrier injury or immunosuppression. Furthermore, colonized patients contribute to environmental contamination and can act as silent vectors for the transmission of VRE to other patients and healthcare personnel.

ICU setting poses a unique challenge in infection control due to the high density of critically ill patients with indwelling medical devices, frequent use of broad-spectrum antibiotics, and invasive procedures. ICU patients are more likely to have prolonged hospital stays, underlying comorbidities, and multiple surgical interventions all of which contribute to increased susceptibility to colonization and infection with multidrug-resistant organisms such as VRE. Environmental persistence of VRE, coupled with inadequate hand hygiene or lapses in standard precautions, can lead to rapid dissemination within ICUs [3-4].

Over the past two decades, the clinical relevance of *Enterococcus* has escalated, primarily due to their increasing resistance to several classes of antibiotics, including β-lactams, aminoglycosides, and glycopeptides [3, 5]. One of the most alarming developments has been the emergence and dissemination of VRE resulting in limited treatment options and has contributed to increased morbidity and mortality in healthcare settings. The resistance is mediated by a group of acquired genes, most notably vanA and vanB, which alter the D-Ala-D-Ala target in the peptidoglycan layer to D-Ala-D-Lac or D-Ala-D-Ser, thereby reducing vancomycin's binding affinity. These genetic elements are often carried on mobile plasmids or transposons, which facilitates horizontal gene transfer not only between Enterococcal species but also to other gram-positive bacteria, including *Staphylococcus aureus*, raising concerns about potential Vancomycin-resistant *S. aureus* (VRSA) strains [6].

Several studies globally have documented varying prevalence rates of VRE fecal carriage among hospitalized patients, particularly in ICUs ranging from 6% to over 30%, with *E. faecium* being the most common isolate [7-9]. In India, studies from tertiary care centres have shown similarly high colonization rates, reflecting the widespread use of empirical broad-spectrum antibiotics and variable infection control measures. These discrepancies in prevalence emphasize the need for localized surveillance data to guide effective infection control practices [9-10].

In this context, screening for VRE colonization plays a vital role in the early identification of potential sources of transmission and in the implementation of appropriate control measures. Surveillance of asymptomatic fecal carriers can provide insights into the burden of colonization, identify associated risk factors, and help prevent downstream infections. Moreover, understanding local patterns of VRE carriage informs hospital antimicrobial stewardship policies and infection control strategies tailored to specific populations and settings. With this background, the present study was undertaken to investigate the frequency of fecal carriage of VRE in patients admitted to the ICU of a tertiary care hospital.

Material and Methods

This cross-sectional study was carried out over a period of six months from January 2024 to June 2024. A sample size of 80 was considered using standard statistical formula, considering the 5% significant level and 10% marginal error. The study was undertaken after obtaining ethical clearance from Institutional Ethics Committee (Protocol number YEC-1/2023/235). Consecutive sampling technique was followed. Non repetitive rectal swabs from patients admitted in surgical ICU for >48hrs were included in the study. Samples that did not yield *Enterococcus* on culture were excluded from the study.

Sample collection procedure: Sample was obtained by inserting a sterile cotton swab 1-2 inches past the anus, rotating it gently for 5-10 seconds against the walls, undertaking necessary universal precautions. The sample was transported immediately to Microbiology laboratory for further processing.

Isolation of Enterococcus: Samples were inoculated on MacConkey agar, incubated at 37°C for 18-24 hrs. Genus *Enterococcus* was identified based on the following characteristics:

(i) Gram positive cocci in pairs – oval/ spectacle shaped
(ii) Magenta coloured pinpoint colonies on MacConkey agar,
(iii) Catalase negative,
(iv) Positive reaction on Bile esculin agar,
(v) Salt tolerance with 6% NaCl.

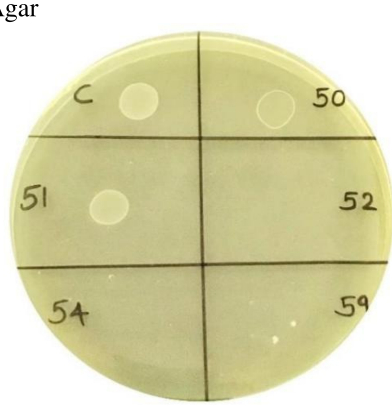
The isolates were stored at -20°C in Nutrient agar vials till the adequate sample size was achieved. Isolates were then retrieved on MacConkey agar and subjected to VRE screening all at once.

Screening for VRE [11]: Preparation of Vancomycin Screen Agar: Brain Heart Infusion (BHI) agar was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes. After autoclaving and cooling to approximately 50°C, Vancomycin was added to the molten BHI agar at a final concentration of 6 μg/mL. The medium was then dispensed into sterile Petri dishes under aseptic conditions and allowed to solidify.

Inoculum Preparation and Inoculation: A bacterial suspension of each *Enterococcus* isolate was prepared in peptone broth and adjusted to 0.5 McFarland turbidity standards by visual comparison. From this suspension, 10μL was inoculated onto the surface of the Vancomycin screen agar using a micropipette and spread gently to form a uniform inoculum spot.

Incubation and Interpretation: Inoculated plates were incubated aerobically at 37°C for 24 hours. After incubation, plates were examined visually for evidence of bacterial growth, which appeared as small colonies or a thin film of confluent growth at the inoculation site as represented in Figure 1. Presence of growth was interpreted as Vancomycin resistance. No growth or a clear inoculation spot was considered indicative of Vancomycin susceptible. All screening tests were performed in duplicate for quality assurance. Appropriate positive and negative control strains were included with each batch of media to ensure reliability of results.

Fig-1: Screening for VRE using Vancomycin Screen Agar



Results

A total of 80 patients admitted to the Surgical Intensive Care Unit were enrolled in this study to evaluate the fecal carriage frequency of VRE.

Demographic Characteristics of study population: Among the study population, 45 patients (56.25%) were male, and 35 patients (43.75%) were female. The age distribution of participants is summarized in Table 1. The majority of patients (45%) were in the 41–60 years age group, followed by 35% in the 61–80 years group, 16.25% in the 21–40 years group, and 3.75% were above 80 years of age.

Table-1: Age Distribution

Age Group (Years)	Number of Patients (n)	Percentage (%)
21–40	13	16.25%
41–60	36	45.00%
61–80	28	35.00%
>80	3	3.75%
Total	**80**	**100%**

Risk Factors: Several risk factors were identified among the study population. Invasive procedures (mostly abdominal surgeries), Polytrauma (multiple surgeries performed), Underlying Malignancy, Diabetes Mellitus, Exposure to multiple antibiotics and long duration of treatment were observed.

VRE Carriage: Out of 80 patients, 7 tested positives for VRE, resulting in an intestinal VRE carriage frequency of 8.75%. The risk factors identified in this group of patients is enumerated in Table 2.

Table-2: Distribution of Risk factors among 7 VRE cases

Multiple antibiotic usage	7 (100%)
Intra-abdominal surgeries	5 (71.4%)
Multiple surgeries due to Polytrauma	2 (28.5%)
Underlying Malignancy	2 (28.5%)
Diabetes Mellitus	1 (14.2%)

Discussion

The present study aimed to determine the frequency of intestinal carriage of VRE using rectal swabs among patients admitted to the surgical ICU for >48 hours. Out of 80 patients screened, the VRE colonization rate was found to be 8.75%. This occurrence, while not alarmingly high, still represents a significant infection control concern in critical care settings where vulnerable patients are at increased risk for subsequent invasive disease. Among the seven VRE colonizers, four patients (57%) were in the age group of 30–50 years, and three (43%) were aged 51–70 years. Our data suggest that in surgical ICU settings, younger individuals may also be at substantial risk, especially when exposed to multiple risk factors [12].

Our findings are consistent with several previous reports. For instance, Ziakas et al. conducted a meta-analysis in U.S. hospitals and reported a VRE carriage rate of 12.5%, while Franyó D et al. from Hungary observed a carriage rate of 5.9% [13, 8]. These values closely align with our results, indicating a moderate level of colonization risk. However, other studies have documented markedly higher rates. Reports from regions such as Pondicherry and Tamilnadu have shown VRE carriage frequencies of 29% and 33% respectively [5, 7]. Another study from Serbia has reported VRE colonization rate of 28.7% [9]. Such variation likely reflects differences in hospital infection control practices, antimicrobial stewardship policies, healthcare infrastructure, and methodological approaches (e.g., direct plating vs. broth enrichment), as well as demographic and clinical characteristics of the study populations. Nevertheless, even moderate levels of colonization can serve as reservoirs for intra-hospital transmission and should prompt active screening and strict adherence to hand hygiene and contact precautions [14].

Common clinical risk factors observed in our VRE-positive patients included multiple antibiotic usage and long duration of treatment resulting in extended hospital stay, invasive procedures, underlying malignancy and diabetes mellitus. These are well-established contributors to gut colonization by multidrug-resistant organisms. Amberpet R et al., have reported significant association between VRE colonization and younger age, prolonged hospitalization, and recent antibiotic exposure [5].

Our study population had received antibiotics such as Ceftriaxone, Amikacin, Piperacillin-tazobactam, Meropenem and Metronidazole as per the hospital antibiotic policy for surgical prophylaxis and treatment of co-existing infections caused by organisms other than *Enterococcus*. Several other studies have implicated Vancomycin and third-generation Cephalosporins as major contributing agents. Antibiotic-induced disruption of the normal gut microbiota creates a niche conducive for resistant organisms like VRE to establish colonization [3-4, 5]. Papadimitriou-Olivgeris M et al observed quinolone administration, chronic obstructive pulmonary disease, chronic renal failure, and number of VRE-positive patients in nearby beds as risk factors for VRE colonization during ICU stay [15].

Conclusion

The present study demonstrates a moderate prevalence (8.75%) of fecal carriage of Vancomycin-Resistant Enterococci among patients admitted to the surgical ICU. However, the study had certain limitations, including the lack of species-level identification and antibiotic susceptibility profiling of the isolates, as well as the absence of molecular characterization of resistance genes such as *vanA* and *vanB*. Additionally, the relatively small sample size and the single-center design may limit the generalizability of the findings.

Future studies involving larger cohorts, multiple centers, and molecular methods, are recommended to provide deeper insights into VRE colonization patterns and resistance mechanisms. The follow-up of patients with intestinal VRE colonization will give more insights into any infections that may develop in the due course of hospital stay.

In conclusion, our findings reinforce the need for continued surveillance of VRE colonization in ICU settings. Risk stratification, judicious antibiotic use, and robust infection control measures remain critical strategies in curbing the spread of Vancomycin resistance within healthcare environments.

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