Original Article

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Microbiology Section

Screening for Intestinal Colonization with Vancomycin Resistant Enterococci and Associated Risk Factors among Patients Admitted to an Adult Intensive Care Unit of a Large Teaching Hospital

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ABSTRACT

Introduction: Gut colonization with Vancomycin Resistant Enterococci (VRE) increases the risk of acquiring infection during hospital stay. Patients admitted in the ICU's are the major reservoirs for VRE colonization due to higher antibiotic pressure.

Aim: To determine the rate of VRE colonization among patients admitted in the Medical Intensive Care Unit (MICU) and to assess the various risk factors which are associated with VRE colonization.

Materials and Methods: This was a prospective study carried out over a period of 18 months from September 2013 to February 2015 in the Jawaharlal Institute of Post graduate Medical Education and Research (JIPMER), Pondicherry, South India. After 48 hours of ICU admission rectal swabs were collected from a total of 302 patients, admitted in MICU. The samples were inoculated on to Bile Esculin Sodium Azide agar with 6mg/L of vancomycin. Vancomycin resistance was confirmed by determination of Minimum Inhibitory Concentration (MIC) by agar dilution method. Isolates were identified up to species level by standard biochemical tests. Vancomycin resistance genes such as *van A, van B and van C,* were detected by Polymerase Chain Reaction (PCR). Risk factors were assessed by multivariate logistic regression analysis.

Results: The rates of VRE colonization in patients admitted to MICU was 29%. Majority of the isolates were *Enterococcus faecium* (77.2 %) followed by *Enterococcus faecalis* (23.8%). All the VRE isolates were positive for *van A* gene. Increased duration of hospital stay, younger age, consumption of ceftriaxone and vancomycin were found to be significantly associated with VRE colonization in MICU. Among VRE colonized patients, six (4.5%) acquired VRE infection.

Conclusion: The rates of VRE colonization in our ICU were similar to other hospitals worldwide. Educating health care workers on the importance of adherence to hand hygiene is essential to bring down VRE colonization rates.

Keywords: ICU, Vancomycin Resistant Enterococci, VRE, *Van A*

INTRODUCTION

Enterococci are common commensals which inhabit the gut of human and animals and in recent decades, have emerged as one of the major causes of nosocomial infections [1]. Vancomycin has been used extensively for the treatment of serious infections due to Enterococci. However, after the first isolate of Vancomycin Resistant Enterococci (VRE) was reported in Europe in1986, there has been a steady increase in the number of VRE infections across the globe [1]. In hospitals, Intensive Care Units (ICU's) are the major reservoirs for antibiotic resistant organisms including VRE. In healthy individuals colonization with VRE is less than 1% but its prevalence is much higher among patients admitted in ICUs [2]. Gut colonization predisposes to subsequent infection resulting in increased cost and morbidity [2,3]. A study from USA described 34 fold increase in the prevalence of VRE colonization in the ICUs between 1989-93 and also reported higher prevalence rate in hospitals with more than 500 beds [4].

A number of risk factors have been associated with VRE colonization in ICU's, the most important ones being extended hospital stay, abdominal surgery and exposure to various antibiotics such as vancomycin, third generation cephalosporins and metronidazole [5-7].

In the present study we determined the rates of VRE colonization among patients admitted in Medical Intensive Care Unit (MICU) and the various risk factors which were associated with VRE colonization. To the best of our knowledge, this is the first report from India describing the VRE colonization rates in ICU patients.

MATERIALS AND METHODS

This was a prospective study carried out over a period of 18 months from September 2013 to February 2015 after obtaining approval from the institute scientific advisory and human ethics committees. A 16 bedded MICU was selected for sample collection. Rectal swabs were collected after 48 hours of ICU admission from a total of 302 adult patients (156 Male and 146 Female). Readmitted patients to MICU were excluded from the study. Additionally samples were collected every 48 hours from 32 patients whose initial VRE colonization was negative. All the negative patients were not included due to financial and time constraints. The samples were processed within two hours of collection and inoculated on to Bile Esculin Sodium Azide Agar (BEA) containing vancomycin 6mg/l, incubated at 37°C for 24-72 hours [7]. Pinpointed black colonies were presumptively identified as Enterococci and were identified up to species level based on Facklam and Collin standard biochemical tests [8]. The Minimum

Inhibitory Concentration (MIC) of vancomycin and teicoplanin was determined for all the enterococcal isolates grown on BEA by agar dilution method, with a MIC of ≥32 mg/L for both vancomycin and teicoplanin being considered as resistant [9]. The susceptibility of the isolates to other antibiotics was also recorded.

The demographic and clinical details of the patient such as age, sex, medical history, clinical diagnosis and co-morbidities such as diabetes, prior hospital admission, date of present admission to hospital and ICU and details of antibiotics administered were documented in a structured proforma. The patients were also followed up for evidence of laboratory confirmed VRE infections during their entire hospital stay.

Molecular detection of vancomycin resistance

PCR for vancomycin resistance genes (*vanA*, *vanB*, *vanC*1, *van C*2/C3) was performed in all the phenotypically vancomycin resistant isolates using published primers as given in [Table/Fig-1] [10].

Extraction of DNA from the bacterial colonies was done using DNA extraction kit (Mericon DNA Bacteria Plus Kit, Qiagen) as per the manufacturer's instructions. Amplification of the DNA was carried out in Mastercycler nexus (Eppendorf) with initial denaturation at 94°C, for 2 min and 30 cycles of denaturation at 94°C for 1 min, annealing temperature at 54°C for 1 min and extension at 72°C for 1min followed by final extension at 72°C for 10 min. The PCR product was analysed on 1.5% agarose gel. DNA ladder 100bp and 1kb was used to compare the size of the PCR amplified product. Electrophoresis was done at 100 V for one hour and gel was viewed under Molecular Imager Gel Doc XR + System (Bio-Rad Laboratories).

STATISTICAL ANALYSIS

Data was analysed using SPSS 20.0 version software. The outcome of colonization with VRE was expressed as binary categorical variable. All categorical variables were analysed using chi-square test while logistic regression was used for continuous independent variables and categorical outcome. Multivariate logistic regression was used for variables with p-value <0.05 using ENTER method. p-value less than 0.05 was considered statistically significant. Difference in difference test was used to analyse the difference between the proportions of VRE positive cases in MICU.

RESULTS

In MICU, out of the 302 patients studied, 83 (27.4%) were colonized with VRE and 219 were negative. Among the 32 VRE negative patients from whom rectal swabs were periodically collected, 5 patients acquired the VRE colonization during the ICU stay. With this, the final VRE colonization rate in MICU increased from 27.4% to 29%. Of the total VRE isolates, 68(77.2%) were identified as *E.faecium* and 20 (23.6%) as *E.faecalis*. The MIC values of all the VRE strains for vancomycin and teicoplanin ranged from 64 to 256 mg/L and all were of *vanA* phenotype. Vancomycin resistance gene *vanA* was detected in all the VRE isolates from colonized patients. None of the isolates were positive for *vanB*, *vanC*1 or *van C*2/C3 genes by PCR [Table/Fig-2].

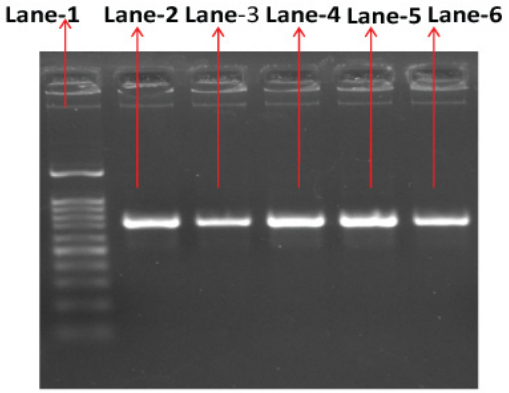
Among the patients admitted to MICU, the risk factors found to be significantly associated with VRE colonization were younger age (for one unit change in the age there will be 3 times less odds of developing VRE colonization), longer duration of hospital stay prior to collection of the specimen (8.20 days among colonized patients vs 4.50 days among those not colonized) as also consumption of vancomycin (p=0.048) and ceftriaxone (p=0.25) [Table/Fig-3,4].

During the study period, five patients from MICU were infected with VRE, 4 among them being previously colonized with VRE. On the other hand, none of the remaining 84 colonized patients developed infection with VRE during their entire hospital stay. The species and antimicrobial susceptibility profiles of the infecting and colonizing isolates were similar in all cases. The average duration between detection of colonization and infection was 6.5 days. The commonest infection was urinary tract infection followed surgical site infections.

All isolates of the VRE from MICU were resistant to teicoplanin. In addition, very high rate of resistance to other antibiotics was noted (88%, 82% and 84% to ampicillin, high level gentamycin and tetracycline respectively). No resistance was observed to linezolid [Table/Fig-5].

Target gene	Primer sequence	Amplicon size
vanA	A1 5'-GGGAAAACGACAATTGC-3'	732 bp
	A2 5'-GTACAATGCGGCCGTTA-3'	
vanB	B1 5'-ATGGGAAGCCGATAGTC-3'	635 bp
	B2 5'-GATTTCGTTCCTCGACC-3'	
*vanC*1	C-1 C1 5'-GGTATCAAGGAAACCTC-3'	822 bp
	C2 5'-CTTCCGCCATCATAGCT-3'	
vanC$_2$/C^3	D1 5-CTCCTACGATTCTCTTG-3	437 bp
	D2 5'-CGAGCAAGACCTTTAAG-3'	

[Table/Fig-1]: Primer sequences used in PCR for vancomycin resistance genes [10].



[Table/Fig-2]: Detection of vancomycin resistant gene *vanA* by PCR (732 b.p).

DISCUSSION

Of all the 19 number of ICU's catering to adult patients in JIPMER, MICU was chosen to represent a heterogeneous population of adults at risk for VRE colonization. The rate of VRE colonization among patients admitted in MICU in the present study was 29%. The rate of VRE colonization in published reports showed a distinct geographic variation with USA reporting higher rates (12.3%) when compared to Europe (2.7%), South America (7%) Asia (5.3%) and Oceania (4.4%) [11]. Although the average rate of VRE colonization in USA hospitals is 12.5% according to the meta analysis of Zakias et al., there were a few hospitals reporting much higher rates of 42% at admission which is even higher than the rates encountered in the present study [11]. The differences in the published rates of VRE colonization may reflect differences in the infection control practices, antibiotic consumption policies, cultural differences among health care personnel and the methodologies followed for detection of colonization [11].

In the meta-analysis by Ziakas et al., the average rate was reported to be 6.3-9% at initial admission while an additional 6.9-11% acquired VRE during their ICU stay [11]. We followed up 32 initially negative patients for subsequent colonization and found 5/32 (15.6%) of them getting colonized which is higher than that reported by Ziakas et al., [11].

Variables	VRE colonisation n=83(%)	VRE not present N=219 (%)	OR (95% -CI)	p-value
Age in years [mean(SD)]	37.9(13.9)	41.6(13.4)	0.979 (0.96-0.99)	0.030
Female	45(30.8)	101(69.2)	0.78 (0.403-1.512)	0.462
Male	38(24.4)	118(75.6)	1	
Urinary catheterization	69(83.1)	192(87.7)	1.49 (0.726-3.076)	0.276
No urinary catheterization	14(16.9)	27(12.3)	1	
Duration of current Hospital stay before specimen collection [Mean(SD)]	8.20(5.2)	4.50(2.9)	1.24 (1.165-1.337)	0.00
H/O Previous hospitalization	12(14.4)	37(16.8)	0.83 (0.410-1.685)	0.608
No H/O Previous hospitalization	71(86.4)	182(84.2)	1	
H/O Surgery	9(10.8)	24(11)	1.49 (0.7263.076-)	0.276
No H/O surgery	74(89.2)	195(89)	1	
Diabetic	14(23.7)	45(76.3)	0.78 (0.403-1.512)	0.462
Non Diabetes	69(28.5)	174(71.5)	1	
Renal failure	9(10.8)	19(8.7)	1.28 (0.554-2.956)	0.563
Non renal failure	74(89.2)	200(91.3)	1	
Malignancy	3(3.6)	10(4.6)	0.78 (0.210-2.921)	0.717
Non malignancy	80(96.4)	209(95.4)	1	
Vancomycin given	49(35.3)	90(64.7)	2.07 (1.23-3.45)	0.006
Vancomycin not given	34(20)	129(79.1)	1	
Ceftriaxone given	64(32)	136(68.0)	2.05 (1.15-3.67)	0.015
Ceftriaxone not given	19 (18.6)	83(81.4)	1	
Metronidazole given	57(26.5)	159(73.5)	0.83 (0.48-1.44)	0.514.
Metronidazole not given	26(30.2)	60(69.8)	1	
Meropenem given	37(28.7)	92 (71.3)	1.11 (0.667-1.848)	0.687
Meropenem not given	46(26.6)	127 (73.4)	1	
Cefoperazonesulbactam given	21(28.8)	52(71.2)	1.08 (0.606-1.952)	0.778
Cefoperazonesulbactam not given	62(27.1)	167(72.9)	1	
Azithromycin given	33(27.7)	86(72.3)	1.01. (604-1.698)	0.961
Azithromycin not given	50(27.5)	133(72.5)	1	
Amikacin given	51(29.7)	121(70.3)	1.29 (0.770-2.163)	0.332
Amikacin not given	32(24.6)	98(75.4)	1	
Levofloxacin given	19(27.1)	51(72.9)	0.97(0.537-1.783)	0.942
Levofloxacin not given	64(27.6)	168(72.4)	1	
Gentamycin given	35(26.3)	98(73.7)	0.90 (0.540-1.500	0.687
Gentamycin not given	48(28.4)	121(71.6)	1	

[Table/Fig-3]: Demographic features and risk factors associated with VRE colonization among patients admitted to MICU (n=302) (Logistic regression analysis).

The risk factors for VRE colonization were assessed based on the previous studies which reported haemodialysis, consumption of

Variables	VRE colonisation n=83(%)	VRE not present N=219 (%)	OR (95% -CI)	p-value
Age in years [mean(SD)]	37.9(13.9)	41.6(13.4)	0.97(0.97-0.95)	0.035
Vancomycin given	49(35.3)	129(79.1)	1.78(1.0-3.15)	0.048
Vancomycin not given	34(20)	90(64.7)	1	
Ceftriaxone given	64(32)	136(68.0)	2.08(1.09-3.97)	0.025
Ceftriaxone not given	19 (18.6)	83(81.4)	1	
Duration of current Hospital stay before specimen collection [Mean(SD)]	8.20(5.2)	4.50(2.9)	1.24(1.16-1.33)	0.001

[Table/Fig-4]: Risk factors associated with VRE colonization among patients admitted to MICU(n=302) {Multivariate (adjusted) Logistic regression analysis}.

Teicoplanin	Ampicillin	High level Gentamycin	Tetracycline	Linezolid
83 (100%)	73 (88 %)	68 (82 %)	70 (84 %)	0

[Table/Fig-5]: Resistance of VRE isolates to other antibiotics (n=83).

vancomycin, use of third generation cephalosporins, exposure to meropenem, increased hospital stay, chronic renal failure, patients with invasive devices, abdominal surgery, bedsores and MRSA co-colonization as being associated with increased chances of VRE colonization [5-7,12-14]. Our study illustrated that younger age group, increased length of hospital stay (8.20 days), consumption of vancomycin and ceftriaxone were significantly associated with VRE colonization in MICU patients. Longer duration of hospital stay prior to being colonized has been found an independent risk factor for VRE colonization. This has been postulated to be due to the higher probable antibiotic exposure in these patients combined with their proximity to an already colonized patient [15].

Although VRE colonization has been found to increase the risk for ensuing VRE infections, this finding is not universal with rates ranging from 0-45% being reported in various studies whereas the risk among non-colonized patients is less than 2% [11]. This wide range may be reflective of the patient population studied as those in general wards are at a lower risk as compared to those with solid organ transplants or hematological malignancies. These differences have also been attributed to variations in the virulence of the colonizing strains [11]. In the present study, 4 of the 88 colonized patients went on to develop VRE infections (4.5%) while only one of the 214 non colonized patients was infected. Based on the same species and similar antimicrobial susceptibility profiles between colonizing and infecting isolates in all the cases we came to the purely speculative conclusion that the same strain went on to cause infection. However molecular tests such as Multi Locus Sequence Typing (MLST) or Pulsed Field Gel Electrophoresis (PFGE) would have confirmed the genetic relatedness between the isolates. In one such study published by Papadimitriou-Olivgeries et al., found 4 out of 107 colonized patients developing infection with the same strain [15]. They made an important observation that VRE colonized patients may also develop subsequent infections with VSE isolates (proved by the same PFGE type).

In this study we looked for the presence of *vanA*, *vanB* and *vanC* genes out of several drug resistance marker genes designated for VRE as *vanA*& *vanB* genes are the most common ones followed by *van C* [1]. All the VRE strains isolated from colonized patients belonged to *vanA* phenotype and it was confirmed by *vanA* gene specific PCR. None of the isolates were positive for *vanB*, *vanC*1 & *vanC*2/C3 genes. There is a global variation between the different van phenotypes and genotypes encountered. In USA and Israel, *vanA* phenotype accounts for majority of the VRE, while in Australia, it is the *vanB* phenotype which predominates [16]. In our own hospital, a few years ago, we had reported both *vanA* and *vanB* phenotypes of VRE although in that study VRE isolates from across the hospital (from both ICU and non-ICU patients) were included [17].

Another potentially deleterious effect of increase in VRE colonization and infection is the risk of developing vancomycin resistance in *Staphylococcus aureus* strains [18]. Vancomycin resistance in *S.aureus* may be acquired by plasmid mediated transmission from glycopeptide resistant Enterococci strains [18].

CONCLUSION

Preventive measures need to be taken especially in ICU's to curtail the spread of vancomycin resistance among Enterococci. Although isolation or cohorting of colonized patients may be ideal, they may not be very practical. Instead strict adherence to hand hygiene and education of health care workers may be more achievable methods of infection control.

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