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A study of clinical outcome, prevalence and molecular characterization of vancomycin resistant enterococci (VRE) at a tertiary care centre

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Abstract: *Background:* Vancomycin resistant enterococci (VRE) has rapidly become one of the leading cause of nosocomial infections and major growing problems in health care facilities globally. *Objectives:* To determine prevalence, perform molecular characterization and to see the clinical outcome of VRE at a tertiary care centre. *Materials and methods:* A total of 122 enterococci isolates collected from various clinical specimens except stool obtained in Clinical Microbiology Laboratory of Rama Medical College Hospital and Research Centre, Kanpur were processed for species differentiation. VRE were detected by MIC determination of vancomycin using agar dilution method. Molecular characterisation of VRE was done for detection of VanA and VanB genes by conventional PCR. Clinical outcome of VRE infected cases were analysed by patient medical records. *Results:* Prevalence of VRE was found to be 6.5% (8/122). (75%) 6/8 were *E. faecium* and (25%) 2/8 were *E. faecalis*. By AST report, among VRE, resistance to teicoplanin was 100%, that to penicillin was 100%, norfloxacin 100%, levofloxacin 100%, high level gentamycin (HLG) 100%, high level streptomycin (HLS) 87.5%, erythromycin 87.5%, ciprofloxacin 87.5%, nitrofurantoin 83.3% and that to linezolid was 12.5%. Gene detection by PCR showed 3 VanA and 1 VanB genes out of 8 VRE and among 5 VRE, VanA or VanB genes were not detected. Mortality was seen in one case of VRE, who had mixed infection with *Klebsiella pneumoniae* and was resistant to linezolid. *Conclusion:* Prevalence of VRE and high level aminoglycoside resistance among them seems high. Prudent use of vancomycin, strict enforcement of infection control policies in hospital and surveillance detection of VRE in hospital should be done regularly.

Keywords: Molecular Characterisation, Prevalence, VRE.

Introduction

Antibiotic resistance shown by various bacteria has become a common problem to health community globally nowadays [1]. According to World Health Organisation (WHO), VRE comes under high priority pathogen in antibiotic resistance for research [2]. Moreover, widespread resistance may hold more consequence for India than for other countries because of India's high bacterial disease burden [3].

More than 60% diseases are infectious in Indian context out of which bacterial diseases are also very common. Mortality rate due to bacterial infections is also more than 10% which is common in ICU [4]. Antibiotic resistance shown by bacteria such as VRE is one of the common cause of morbidity and mortality in India [2, 5]. Treatment options and effective antimicrobial agents for VRE are often limited and the possibility of the transfer of vancomycin-resistant genes to other Gram-positive microorganisms also remains [6].

Despite many studies in other countries and only a few studies in certain parts of India [6-9], no study has been done to observe prevalence of VRE and their molecular characterization in Kanpur (Northern India) tilldate. This study will include; determination of prevalence of VRE, reporting of antibiotic

susceptibility test, detection of high level aminoglycosides resistance among them, species differentiation of isolates, detection of genes responsible for vancomycin resistance, and to see the outcome of VRE infections. So, this study seems essential to medical and health community of India for infection control in hospital settings as well as in community. Findings of this research study could be highly beneficial for formulating antibiotic policy in the hospitals in and around Kanpur.

Material and Methods

After obtaining ethical approval from Rama Medical College Hospital- Research & Ethical Sub Committee, a prospective cross sectional study was done. Sample size was calculated by following formula [10]:

Sample size (n) = $\frac{2(p)(1-p)\left(Z\beta + \frac{Z\alpha}{2}\right)^2}{d^2}$

where, $\frac{Z\alpha}{2}$ = It is standard normal variate [at 5% type I error ($p<0.05$) it is 1.96]

Zβ= Power of test. It is 0.84 for 80% power.

p = Expected proportion in population based on previous study. According to previous studies, it may not more than 8%. (7) So it is 0.08

d= Expected difference of incidence from previous studies. So it is 0.1

Now, Sample size = $\frac{2(.08)(.92)(0.84+1.96)^2}{(01)^2}$ = 116.

According to sample size calculation, a total of 122 Enterococci isolated from various clinical specimens, excluding stool, during November 2017- May 2019 (18 months duration) were included in the study for determining prevalence of VRE. Identification of Enterococci was done by colony characters, Gram positive cocci in Gram staining, catalase test negative, hydrolysis of Bile esculin, tolerance to 6.5% NaCl, and motility test report [11]. Species differentiation of Enterococci was done according to Facklam and Collin's classification [12]. Antibiotic susceptibility test was done by Kirby Bauer's disc diffusion method according to standard guidelines [13]. *E, faecalis* ATCC 29212 was used as control. AST report was used to detect high level aminoglycoside (HLG; 120 mcg & HLS 300 mcg) resistance also. Zone diameter equal or less than 6 mm for both was considered as high level aminoglycoside resistance (HLAR) [14]. Detection of vancomycin resistant Enterococci (VRE) was done by agar dilution method according to CLSI guidelines [15]. MIC of vancomycin equal or greater than 32 mcg was considered as VRE.

Among VRE, detection of vancomycin resistant genes, with special reference to VanA and VanB, was done using conventional PCR according to standard guidelines [16]. Primers used for VanA and VanB include; Forward 5'-GGGAAAACGACAATTGC-3' & Reverse 5'-GTACAATGCGGCCGTTA-3' for VanA and Forward 5'-ACGGAATGGGAAGCCGA-3' & Reverse 5'-TGCACCCGATTTCGTTC-3' for VanB respectively [7,17]. *E. faecium* ATCC 700221 was used as Positive control for VanA and *E. faecalis* ATCC 51299 was used as positive control for VanB.

Results

Out of 3,438 samples obtained for culture and sensitivity within 18 month's duration in Clinical Microbiology laboratory, 872 were culture positive. Among these 872 culture positive cases, 122 (13.9%) were identified as enterococci. Prevalence of VRE was determined by agar dilution method and it was found to be 6.5% (8/122) [Figure-1(a), Figure-1(b)]. 75% of VRE were isolated from urine sample, 12.5% from blood and 12.5% from pus. Total of 75% VRE were isolated from female cases and 25% VRE were isolated from male cases.

Fig-1(a): Pie chart showing prevalence of VRE.

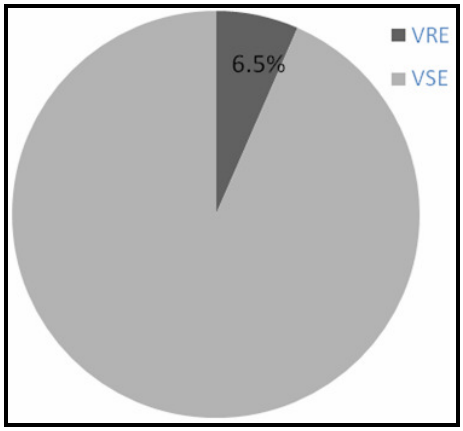
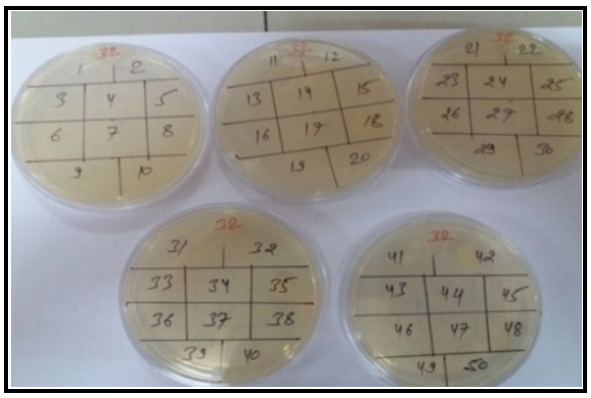


Fig-1(b): Showing MIC of vancomycin = or > 32 by agar dilution method among VRE.



Total 87.5% of VRE cases were isolated from patients having > 60 years age group and 12.5% of VRE cases were isolated from 31-40 years age group. There was no seasonal influence for prevalence of VRE. Total 50% VRE were isolated from ICU followed by 25% from medicine ward and 25% from surgery ward. All (100%) VRE were isolated from inpatients. Among 8 VRE, 75% were *E. faecium* and 25% were *E. faecalis*.

Table-1: Showing AST pattern of VRE

Name of antibiotics	Sensitive- No. /8 (%)	Resistant- No. /8 (%)
Vancomycin	0 (0%)	8 (100%)
Teicoplanin	0 (0%)	8 (100%)
Penicillin	0 (0%)	8 (100%)
High level gentamycin	0 (0%)	8 (100%)
Norfloxacin	0/6 (0%)	6/6 (100%)
Levofloxacin	0/1 (0%)	1/1 (100%)
Ciprofloxacin	1 (12.5%)	7 (87.5%)
High level streptomycin	1 (12.5%)	7 (87.5%)
Erythromycin	1 (12.5%)	7 (87.5%)
Nitrofurantoin	1/6 (16.7%)	5/6 (83.3%)
Tetracycline	5 (62.5%)	3 (37.5%)
Linezolid	7 (87.5%)	1 (12.5%

Out of 8 VRE, according to AST report, resistance to teicoplanin was 100%, that to penicillin was 100%, norfloxacin 100%, levofloxacin 100%, HLG 100%, HLS 87.5%, erythromycin 87.5%, ciprofloxacin 87.5%, nitrofurantoin 83.3% and that to linezolid was 12.5% [Table-1]. Out of 8 VRE, 3 samples showed presence of VanA or VanB genes & 5 samples didn't show VanA or VanB genes [Figure -2(a), 2(b), and 2(c)].

Fig-2(a): Showing L= ladder (1000 bp) in the centre, PC-1=Positive control for VanA (732 bp) in left, and PC-2=Positive control for VanB (647 bp) in the right to the ladder.

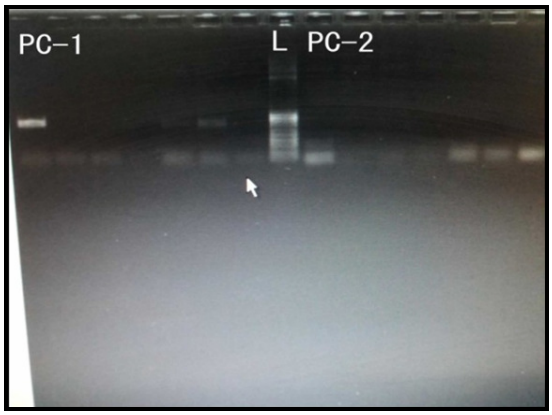


Fig-2(b): Showing VanA and VanB genes detected among VRE & ladder in the centre. L=ladder. 1,2,3=VanA positive, 4= VanB positive.

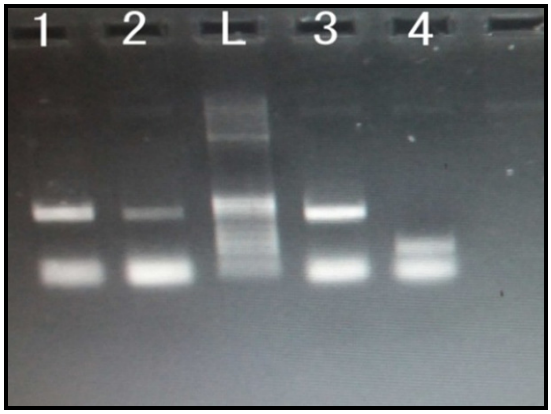


Fig-2(c): Showing no VanA and VanB genes along with ladder kept in the centre. L=ladder, and 5,6,7,8=negative for VanA & VanB.

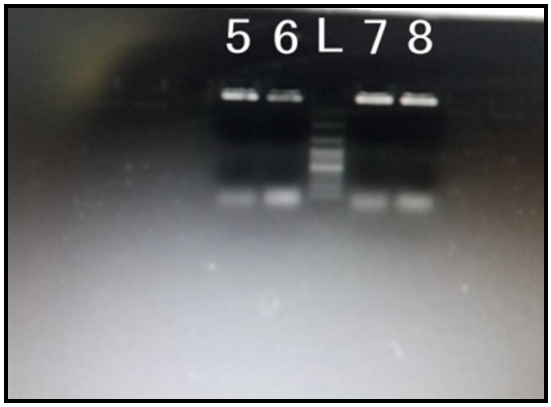


Table-2: Showing genes detected among VRE with special reference to VanA & VanB.	
Gene/s	**Number of VRE (%)**
VanA & VanB	1/8 (12.5%)
VanA	2/8 (25%)
genes other than VanA or, VanB	5/8 (62.5%)

1 sample showed both VanA & VanB genes, 2 other samples contain VanA genes & other 5 VRE contain genes other than VanA or VanB [Table-2]. Outcome of the study showed one VRE infected patient died who had Linezolid resistance also in addition to *Klebsiella pneumoniae* infection who was getting treated with colistin. All other VRE infected patients were successfully treated with Linezolid [Table-3].

Table-3: Showing clinical outcome of VRE infection	
Clinical outcome	**Number of VRE (%)**
Death	1/8 (12.5%)
Treated with linezolid	7/8 (87.5%)

Discussion

Enterococci are one of the causative agents of various clinical infections such as UTI, bacteraemia, skin infections etc [18]. Prevalence of enterococci was found to be 13.9% in our study. Few other studies report prevalence of enterococci to be 3.5% in Ethiopia and 5.9% in Nigeria [19-20]. This similarity or difference in findings might be due to geographical location, isolation technique for bacteria in various hospital set-up and disease pattern in particular places [21].

Disc diffusion method is not considered standard method to determine prevalence of VRE and dilution method is gold standard [15]. Among dilution methods, agar dilution is done for convenience of the test [15]. Our study showed prevalence of VRE to be 6.5% by agar dilution method which is similar to findings of few studies on nearly places of Kanpur [7, 22]. However, dissimilar findings also had been reported by other studies in South India [6, 8-9]. Similarity or difference in hospital antibiotic policies in these places might be the reason for different prevalence of VRE in those areas. Based on samples, prevalence of VRE was found to be highest (75%) among urine samples followed by blood and pus (12.5% each). Similar reports had been published in various studies [6-9]. As urine was the most common sample obtained for culture and sensitivity followed by blood and pus in this study, this could be the reason for such findings.

Based on gender, prevalence of VRE was found to be high among females (75%) compared to males (25%) in our study. As females were infected more with enterococci compared to males as more urine samples were obtained during study and UTI is more common among females, this could be the reason for such finding in our study. Based on age, prevalence of VRE was found to be high among old age grouped people. In this study, people with age group above 60 (61- above 80 years) were mostly (87.5%) infected with VRE. Remaining 12.5% VRE were isolated from people age ranging from 31-40 years. As VRE are opportunistic pathogen and is more common among old aged people with immunocompromised status, this could be the reason for such finding.

Prevalence of VRE was found to be independent on month or season in our study. Similar report had been found in other studies also [6-9]. However, 50% VRE were isolated in the summer season. This could be due to more number of enterococci isolated in this season. Sourcewise, mostly (50%) VRE were isolated from ICU, 25% from medicine ward and 25% from surgery ward. Overall, all (100%) VRE were isolated from inpatients [6-9]. As serious patients are admitted in ICU who are more exposed to various antibiotics, so this could be the reason for high isolation rate of VRE from such patients.

As hospitalised patients are mostly immunocompromised and enterococci are normal gut flora which causes endogenous infection among immunocompromised people, there is high chance of getting infected with drug resistant bacterial infections. AST pattern of VRE showed that all VRE (100%) were resistant to teicoplanin also. Further, 12.5% were resistant to linezolid

also. This has alarmed to medical community for searching another suitable antibiotic to linezolid resistant bacterial infections. Despite discovery of quinupristin and dalfopristin, as these drugs should be kept in reserve for MDR VRE infection cases, time has come to think regarding prudent use of new antibiotics against VRE and drug resistant bacteria. Moreover, resistance of VRE to HLG was found to be 100% and that to HLS was 87.5% in this study. Reports of few other studies show similarity or variation to this finding [22-25].

This showed that HLG and HLS also has limited role in case of VRE infections. This is the biggest challenge to medical community globally. In addition, we found 100% resistance to levofloxacin for treatment of bacteremia or septicaemia and 83.3% resistance to nitrofurantoin for treatment of UTI with reference to VRE which is another concern for medical community. Various studies had shown that, among various species of enterococci, VRE are mostly *E. faecium* followed by *E. faecalis* [6-9]. In our study, we also found similar report. Among VRE, 75% were *E. faecium* and 25% were *E. faecalis* in our study. As *E. faecium* is common species of enterococci which is vancomycin resistant, sometimes *E. faecalis* also has been found to be responsible for vancomycin resistance [7].

In our study, we detected 2 VanA genes, 1 VanA and VanB genes, and 5 other VRE didn't show VanA or VanB genes. Detection of VanA and VanB both genes in same VRE could be due to teicoplanin resistance along with use of vancomycin in past [26]. We could not detect gene responsible for VRE among 5 VRE cases as detection of genes other than VanA or VanB was not kept in mind during study plan which is limitation of our study. As linezolid is drug of choice for treatment of VRE infections, almost all (7/8) VRE were successfully treated with linezolid. However, 1 VRE was resistant to linezolid which had mixed infection with *Klebsiella pneumoniae* and was getting treated with colistin died in our study. It was not possible to declare whether that case died due to VRE or colistin resistant *Klebsiella pneumoniae* infection.

Conclusion

Almost all VRE were sensitive to linezolid except one case. Even that can be treated with quiniprustin and dalfopristin. However, this is a challenge to medical community as these drugs should be kept in reserve for future. So, prudent use of vancomycin, strict enforcement of infection control policies in hospital, and surveillance detection of VRE in hospital should be done regularly.

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