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Original Research Article

Genotypic Characterization of Van A and Van B Genes among Vancomycin Resistant *Enterococci* Isolated from Urine Samples

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Abstract:

Infections caused by VRE are difficult to treat because different gene clusters are known to confer Vancomycin resistance. Van A and Van B genes are transferable and clinically relevant. This study aimed to identify Vancomycin resistant genotypes in strains causing U.T.I. Out of 250 *Enterococci* 37 (14.8%) of the isolates were Vancomycin Resistant *Enterococci* (VRE) of which 17(45.94%) were from male and 20 (54.05%) were from female Patients. Total species distribution in VRE isolates was 3(8.1%), 31(83.78%) and 3(8.1%) for *E. faecalis*, *E. faecium*, other sps. Antibiotic resistance profile of VRE isolates was evaluated. All the VRE strains were 100% resistant to Ampicillin whereas 89.18% VRE strains were resistant to Ciprofloxacin and 78.3% VRE strains were resistant to High Level Gentamicin. Van A and Van B genes were detected in 14(37.8%) and 1(2.7%) of strains respectively. The present study showed prevalence of Van A and Van B genes carrying Enterococcus in urinary isolates.

Keywords: VRE, *E. faecalis, E. faecium*.

Introduction

Enterococci are commensal flora found in the colon, but they can cause significant infections in other parts of the body, most notably the urinary bladder [1]. Enterococci demonstrate intrinsic and acquired resistance to a wide range of antibiotics [2]. A glycopeptide antibiotic Vancomycin is considered the 'last resort' for therapy and is used exclusively in case of Gram-positive infections where there is resistance to Penicillin and Cephalosporins [3].

Antimicrobial resistance (AMR) has emerged as a serious danger to human health worldwide. AMR develops when bacteria develop resistance to counteract the effects of antimicrobials. As a result, AMR jeopardizes infection therapy, increasing morbidity and mortality. Vancomycin, a glycopeptide antibiotic, functions by inhibiting bacterial cell wall synthesis. *E. faecium, E. faecalis*, and many other *Enterococcus* species have developed resistance to Vancomycin. *Enterococci* develop vancomycin resistance by mutations and/or the acquisition of external genetic material that confers resistance [4]. Various genes, including VanA, VanB, VanD, VanE, VanG, and Van L, have been shown to contribute to vancomycin resistance in *Enterococci* [4].

India has one of the highest burdens of infectious diseases in the world and is among the countries with the biggest AMR burden in both humans and animals [5] the current study aimed to genotypically detect the VanA and VanB genes in urinary isolates of Enterococci using PCR in VRE isolates.

Material and Methods:

This cross-sectional study included *Enterococci* isolated from urine samples of suspected UTI cases in Microbiology laboratory, SMS Medical College, Jaipur from June 2022 to December 2023.

Permission to conduct the study was obtained from the Institutional Ethics Committee. *Enterococci* were identified presumptively by colony morphology, Gram staining and confirmed by biochemical reactions including Catalase and Hydrolysis of Bile esculin as per standard lab protocol. Antimicrobial susceptibility testing was performed using Kirby Bauer Disc Diffusion Method as per CLSI guidelines 2021.VRE were subjected to detection of Van A and Van B genes. The presence of Van A and Van B genes was detected by Polymerase Chain Reaction (PCR). Presence of the genes encoding the Vancomycin-

resistance determinants were investigated by using specific primers (Table 1). Bacterial DNA was extracted from isolates by boiling DNA Extraction method [6].

Table 1: Primers used for PCR for detection of Vancomycin resistant genes

Target Product	Oligonucleotide Sequence	Amplicon Size
Van A	5'CATGAATAGAATAAAAGTTGCATTA 3'	1030 Base Pair
	5' CCCCTTTAACGCTAATACGATCAAA3'	
Van B	5' GTGACAAACCGAGGCGAGGA 3'	433 Base Pair
	5'CCGCCATCCTCCTGCAAAAAA 3'	

ATCC 51299 was used as positive control. PCR reactions were carried out in 25µl volume in micro centrifuge tube using 13µl commercially available, ready to use master mix, 1µl of each forward and reverse primer, 7µl of molecular grade water and 3µl of DNA template. DNA amplification was done in a PCR thermocycler(BIO-RAD) with Specific thermal cycling profile and was programmed for Pre denaturation step at 95°C for 4 minutes followed by 30 cycles of Amplification(95°C for 30 seconds, 52°C for 60 seconds and an Extension step at 72°C for 60 seconds).

PCR products were resolved on 1 percent Agarose gel stained with Ethidium bromide. A 100 Base Pair DNA ladder (Takara master mix) was run in every gel and the size of each VRE genotype was determined by the size of amplified product.

Results

A total of 250 *Enterococci* isolated from urine samples were included in the study out of which 183 were from IPD and the rest 67 from OPD. Out of 250, 37 (14.8%) of the isolates were Vancomycin-resistant *Enterococci* (VRE). In 37 VRE isolates 17(45.94%) were from male and 20 (54.05%) were from female patients. Species distribution in Vancomycin Resistant isolates was 3(8.1%), 31(83.78%), and 3(8.1%) for *E. faecalis* (8.1%), *E. faecium* (83.78%), other sps (8.1%). All the strains were (100%) resistant to Ampicillin whereas (89.18%) strains were resistant to Ciprofloxacin and (78.3%) strains were resistant to High Level Gentamicin. Van A and Van B genes were detected in 14(37.8%) and 1(2.7%) of VRE strains respectively (Fig1) and (Fig 2).

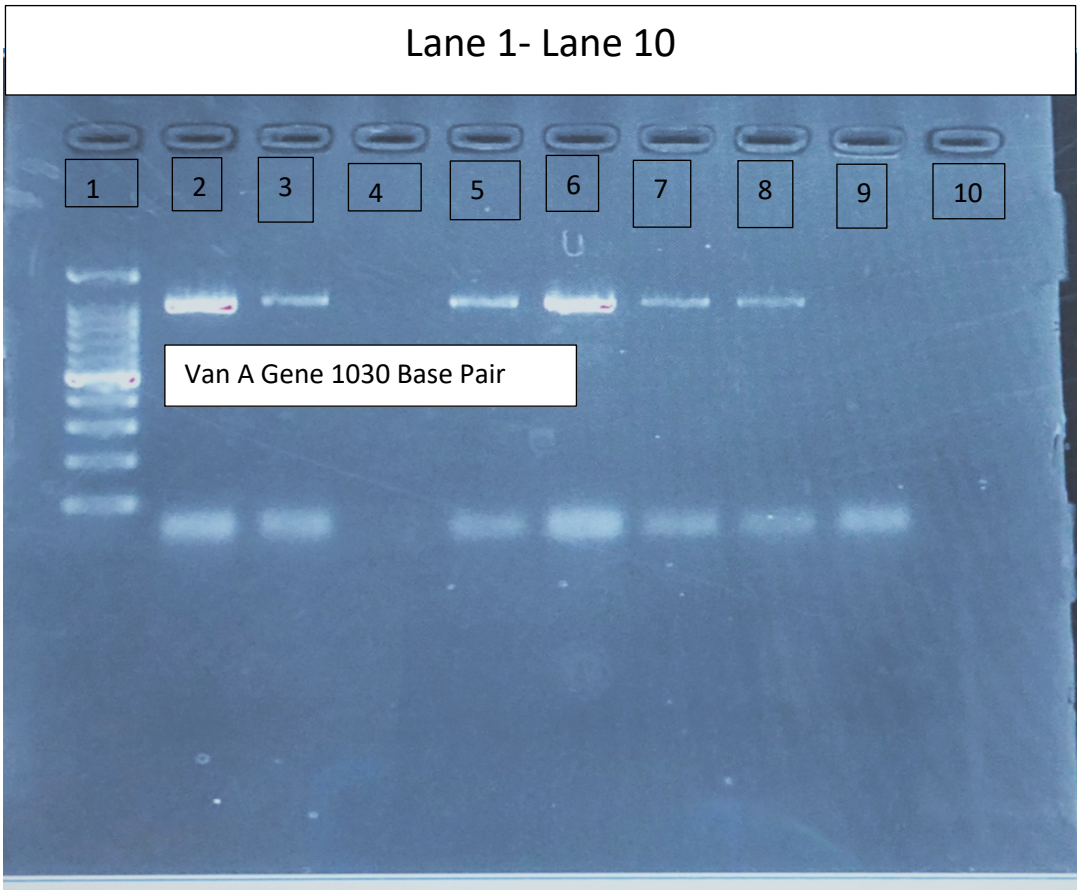

Figure 1: Van A Gene: PCR Analysis of DNA from Vancomycin Resistant *Enterococci*

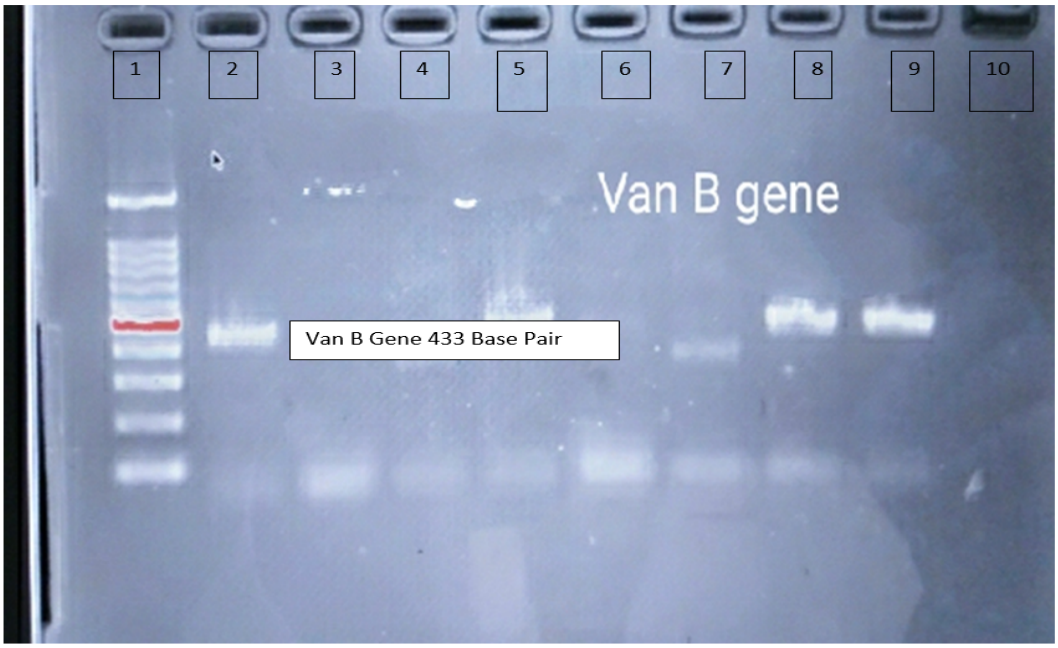

Figure 2: Van B Gene: PCR Analysis of DNA from Vancomycin Resistant *Enterococci*

The Results were analyzed by visualization in Gel Electrophoresis Image, indicating the presence of Vancomycin resistant genes in tested isolates. Here's a summary of findings for Fig1 and Fig 2.

Lane 1: 100 Base Pair DNA Ladder

Lane 2: Positive Control

Lane 3-Negative control

Lane 4-Lane 10: Test strains

Discussion

Enterococci are significant pathogens causing Urinary tract infections. These bacteria show vulnerability to VRE acquisition and dissemination. In the present study genotypic detection of Vancomycin Resistant Genes (Van A and Van B) was done via Multiplex PCR. A total of 250 Enterococcal isolates were included in the study out of which 183 were from IPD and rest 67 were from OPD similar results were reported by Saraswathy MP et al 36% isolates were from OPD and 64% from IPD [7]and in study done by Sharma K et al 29% were from OPD and 70.8%. Were from IPD [8]. Enterococcal prevalence in healthcare settings can be attributed to the bacterial ability to survive on multiple surfaces for long periods of time and in harsh, heavily disinfected environments due to which rate of infection is more in Indoor Patients than outdoor Patients [Codelia-Anjum A et al 9].

In the present study 14.8% of isolates were Vancomycin resistant which was almost similar to the study done by Das A et al[10] i.e.,18.4%. While Toner L et al [11] reported Vancomycin resistance in 9.8% of strains. Also, Mussadiq S et al [12] reported 8.6% of Urinary isolates as VRE.

Out of 250 urinary isolates 37 were Vancomycin-resistant *Enterococci* (VRE) of which 17(45.94%) were from males and 20 (54.05%) were from female patients. These findings were in agreement to studies done by Eltayeb et al [13] and Toner L et al [11] with VRE more common in females. No gender predilection for V.R.E. was observed.

In present study maximum VRE isolates were from age group that is 0-12 years as VRE seems to be higher in children in Lower age groups which could be because of prolonged hospitalization, immunosuppression, low birth weight, antibiotic intake [Shirvani F et al 14].

Among Vancomycin Resistant Enterococci (VRE) strains, E faecium species was significantly higher (83.78%) than the *E. faecalis* (8.10%) which is in agreement to results of Das S et al[15] who reported E faecium (56.%) and *E. faecalis* (43.09%) and Meena S et al [16]who found E faecium (96.15%) and *E. faecalis* (3.8%). The reason for this resistance pattern as stated by X Zhou et al [17] is that E. faecium is persistently inherent to build resistance to antibiotics and environmental stressors that allows the species to thrive in hospital environments. Antimicrobial susceptibility pattern of VRE shows that all VRE strains were 100% resistant to Ampicillin, these results were in concordance to study done by Das S et al [15] who reported 100% resistance to Ampicillin while Zhanel et al[18] recorded 86% resistance in VRE isolates. This High-level resistance to Ampicillin in E. faecium is mainly

due to the enhanced production of PBP5 and/or by polymorphisms in the beta subunit of this protein. The dissemination of high-level Ampicillin resistance can be the result of both clonal spread of strains with mutated pbp5 genes and horizontal gene transfer [Gagetti P et al19].

In present study (89.18%) isolates were resistant to Ciprofloxacin which is similar to study done by Das A et al [10] who recorded 74.7% strains resistance to Ciprofloxacin contrasting to results of Aleyasin A et al [20]who recorded 41% of resistance. In the current study 78.3% of VRE were resistant to High level Gentamicin. Resistance to aminoglycosides, especially high-level aminoglycoside, abolishes the synergy between cell-wall-active agents and aminoglycosides [V. Sharifzadeh Peyvasti 21].

Gentamicin resistance is predominantly the result of the presence of the inactivating enzyme 2"-phosphotransferase-6′-acetyltransferase conferring resistance to gentamicin, tobramycin, netilmicin, amikacin, and kanamycin. Hence, Gentamicin resistance is a good predictor of resistance to other aminoglycosides [Cetinkaya et al 22]. Genetic Lineages of *E. faecalis* and E. faecium exhibit conserved genomes, although they possess a significant accessory genome (up to 38% in E. faecium), which contributes to remarkable genomic plasticity. The evolution of *Enterococci* was predominantly influenced by recombination and proficiency in acquiring novel genes through HGT(horizontal gene transfer)facilitated by MGEs(mobile genetic element) such as plasmids, transposons genomic islands (GI), and prophages [Marques J et al 23].

The antimicrobial resistance profiles of E. faecalis and E. faecium show significant differences for certain antibiotics. Both species exhibit 100% resistance to Ampicillin and Vancomycin, with statistically significant differences indicated by a P-value of 0.009 out of total VRE isolated.

Notably, High-Level Gentamicin (HLG) resistance is significantly higher in E. faecium (77.4%) compared to *E. faecalis* (33.3%), with a P-value of 0.022. Similarly, Ciprofloxacin resistance is observed in 90.3% of E. faecium and 100% of *E. faecalis*, also showing a significant difference (P-value 0.020). Other antibiotics, including Amikacin, Tetracycline, and Linezolid, show varying resistance patterns, but these differences are not statistically significant. Overall, E. faecium demonstrates higher resistance rates, particularly for HLG and Ciprofloxacin.(Table 2). Overall statistically significant difference was observed between two groups for Ampicillin, Vancomycin, High level Gentamicin and Ciprofloxacin.

Table 2: Resistance profile of Vancomycin Resistant *Enterococci* Antibiotics

Antimicrobial/or resistant phenotype (disc strength in µg)	*E. faecalis*(n=3)	*E. faecium*(n=31)	P value (chi-square test)
Ampicillin	3(100%)	31(100%)	0.009
Teicoplanin	_	3(3.2%)	0.673
Amikacin	1(33.3%)	1(33.3%)	0.938
Vancomycin	3(100%)	31(100%)	0.009
High Level Gentamicin	2(33.3%)	24(77.4%)	0.022
Ciprofloxacin	3(100%)	28(90.3%)	0.020
Cefotoxin	_	2(3.22%)	0.938
Doxycycline	_	3(3.2%)	0.673
Tetracycline	1(33.3%)	11(32.2%)	0.223
Lineozolid	_	7(3.2%)	0.216
Levofloxacin	_	2(6.45%)	0.938
Nitrofurantoin	_	10(3.22%)	0.097
Fosfomycin	1(33.3%)	12(38.7%)	0.175

Van A and Van B genes are commonly responsible for Vancomycin Resistance. Van A is more prevalent than Van B.

In the present study Van A gene was reported 14(37.8%) and Van B 1(2.7%) of VRE strains and these results were in agreement to study done by Bhat NR et al in which Van A was reported 4% and 1.33% isolates in Van B [24]. Das A et al [25] recorded Van A in 75% and Van B in 25%. So all these studies show Van A Gene is more common than Van B. which could be because Van A gene is associated with clinical strains, and the fact that patients have received Vancomycin for a long time justifies the presence of Van A gene in VRE whereas Van B gene is clustered and occupies a larger chromosomal region, and the possibility of transmission among strains is low. The Van B gene is primarily associated with food contamination and epidemics [Moosavian et al 26].

Conclusion

Vancomycin resistant *Enterococci* (VRE) is an important urinary pathogen associated with Multi Drug Resistance to commonly used antibiotics.

Study of Resistant Gene helps in understanding the prevalence of Antimicrobial resistance (AMR) in VRE and guides clinicians in selecting appropriate therapy thereby reducing morbidity and mortality.

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