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Identification of Vancomycin Resistance Determinants in Twin Cities of Rawalpindi/Islamabad

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Abstract

Vancomycin resistant Enterococci have emerged rapidly in the recent years leading to treatment failure. The aim of this work was to identify vancomycin resistance determinants; *vanA* and *vanB* genes in Enterococci. The blood, urine and throat samples were collected from 150 patients from local hospitals of twin cities of Rawalpindi/Islamabad. Forty-nine phenotypically confirmed isolates were further confirmed by PCR amplification for vancomycin determinants (genes for *vanA* and *vanB*) for vancomycin resistant *Enterococci*. Dependence and frequency distribution of VRE and VSE bacteraemia with respect to age, gender and source was also studied. Phenotypically resistant strains were positive for *vanA* while negative for *vanB*. vancomycin susceptible enterococci (VSE) could be isolated more from urine samples as compared to blood whereas vancomycin resistant enterococci (VRE) was found more prevalent in the blood samples (*p* value= 0.000). VRE was more frequently isolated from patients aged 50 or above whereas VSE prevalence was same in both age groups (*p* value=0.002). Gender was not found to have any significant impact on VRE or VSE bacteraemia. This study reports *vanA* gene cluster responsible for resistance in Pakistani population and frequently isolation of VRE from blood samples.

Key words: *Enterococcus*, Vancomycin resistant Enterococci, Pakistan

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1. INTRODUCTION

The Gram-positive *Enterococci* are present as gastrointestinal tract normal microflora in both healthy humans and animals. These have the ability to colonize wounds and ulcers in hospitalized people. *Enterococci* are attributed as second major cause of bloodstream infections (BSI) the third for hospital acquired infections (HAI)[1]. About 14% of HAI have reportedly been caused by Enterococci[2]. More than 40 species have been identified as part of *Enterococcus* genus, however *E. faecium* and *E. faecalis* have emerged as major cause of human infections comprise 75% and 20% of human infections, respectively[3-4]. A recent Chinese study reported *E. faecium* as compared to *E. faecalis* that accounted for more bloodstream infections with 24% mortality rate in total[5]. Emergence of moderate to high level drug resistance has been attributed for the failure of eradication therapy in enterococcal infections[2].

The emergence of multi drug resistant (MDR) enterococcal species is attributed to both horizontal and vertical transfer of resistant determinants. Enterococci became resistant to glycopeptides (vancomycin and teicoplanin) by acquiring genes through plasmid or transposon that enabled bacteria to bypass antibiotic susceptible critical steps in cell wall formation[6]. The intrinsic resistance of enteroccci against β-lactam antibiotics and such transposon mediated horizontal transfer of vancomycin resistance genes have limited the use of these antimicrobials for treating VRE.

Since its first use in 1958, vancomycin has been used for the treatment of Gram positive bacterial infections as it effectively blocks the cell wall formation by targeting its building blocks[7]. The resistance determinant against vancomycin is a gene complex that expresses to synthesize the abnormal precursor of peptidoglycan to which vancomycin binds with very low affinity. *vanA* and *vanB* being the dominant genes in clinically important enterococcal species (*E. fecalis* and *E. faecium*) are present on mobile elements that jump across species to confer resistance. Employing these jumping genes, VRE can horizontally transfer its resistant determinants to VSE and other organisms like Gram positive *S. aureus*[8-9].

High level resistance with MIC of more than 64 µg/ ml against vancomycin and moderate resistance with MIC of over 16 µg/ ml against toteicoplanin is conferred by *vanA*[10]. The resistance level (MICs, 4->256 mg/ml) in *vanB* is lower than *vanA* and is attributed to VanB ligase enzyme that converts D-Ala-D-Ala to D-Ala-D-Lac, perhaps due to less substitution[11]. According to geographic distribution reports about *vanA* and *vanB* genotypes, *vanA* is frequently found in Europe, most US states and in most countries of Asia[12]. The gene cluster reported for both *vanA* and *vanB* is of 1.3 kb (www.ncbi.nlm.nih.gov).

The major objective of our study was to ascertain the resistance determinants frequently found in Pakistan that confer resistance to *E. faecalis* and *E. faecium* and also analyze significance of the clinical data of patients for occurrence of VRE or VSE bacteraemia.

2. MATERIALS AND METHODS

2.1 Patient's clinical data

Data including age of the patients (divided into two groups of upto 50 years and above 50 years), gender, history of vancomycin treatment, surgical procedures and current medication profile was collected from the hospital record.

2.2 Clinical Isolates of Enterococcus

The blood, urine and throat samples were collected from 150 patients of local hospitals providing tertiary care to the patients of twin cities of Rawalpindi/Islamabad during seven months of the study period. Written consents were taken from the patients and guardians before sampling and study was approved from concerned bodies. Forty nine clinical isolates of *Enterococcus* were identified using biochemical tests. Among these were 13 vancomycin resistant *E. faecium*, 11 vancomycin sensitive *E. faecium,* 5 vancomycin resistant *E. faecalis*, and 20 vancomycin sensitive *E. faecalis*. Disc diffusion was used to test antibiotic susceptibility testing and MIC recorded. Plates of Azide Dextrose agar were used to maintain vancomycin susceptible strains and those of nutrient agar with 60 µg/ml vancomycin were used for vancomycin resistant strains.

2.3 Antimicrobial Susceptibility Testing

Plasmid Extraction

Luria Bertani (LB) broth was used to grow desired bacterial strain and growth was harvested during logarithmic phase (O.D600 of 0.6). Plasmid Extraction Kit (Invitrogen, Life Technologies) was used to isolate high copy number of plasmid. The plasmid obtained was checked using 1% agarose gel.

Primer Designing

Primers were designed for both *vanA* and *vanB* genes. The gene sequences were obtained from NCBI. Oligocalc program was used to analyze the GC content, Tm and complementarity properties of both primers. BLAST and ClustalW programs were used for specificity and proper alignment, respectively. Primer set for *vanA* included: Forward primer: 5-GAGGAGCATGACGTATCGGTA-3 and reverse primer: 5-

CGATCAAGCGGTCAAT CAGT-3. The primer set for *vanB* included; Forward primer: 5-GTTGCTCGGAGGAACATGAT-3, and reverse primer: 5-GATAGCGGCTGTACGATGTA-3.

2.4 Molecular Identification

PCR amplification was carried out for *vanA* and *vanB* genes using the respective primers. PCR profile included denaturation initially at 94°C for 4 minutes that was followed by 35 cycles of denaturation, annealing and elongation (94°C for 45 seconds, 55°C for 1 minute and 72°C for 2 minutes). Final elongation for 7 minutes was done at 72°C. The resultant PCR product was analyzed on 1% agarose gel and 1 kb ladder for size estimation.

2.5 Statistical Analysis

SPSS 16.0 was used for data analysis and graphs were made with GraphPad Prism 5.0 software. The significance of data was analyzed using Chi-square test.

3. RESULTS AND DISCUSSIONS

3.1 Presence of vanA in VRE

The primers for *vanA* and *vanB* gene cluster gave the desired PCR amplicon of approximately 936 bp for all VRE isolate (5 vancomycin resistant *E. faecalis* 13 and vancomycin resistant *E. faecium* (Fig. 1) that confirmed the resistant nature of the isolates.

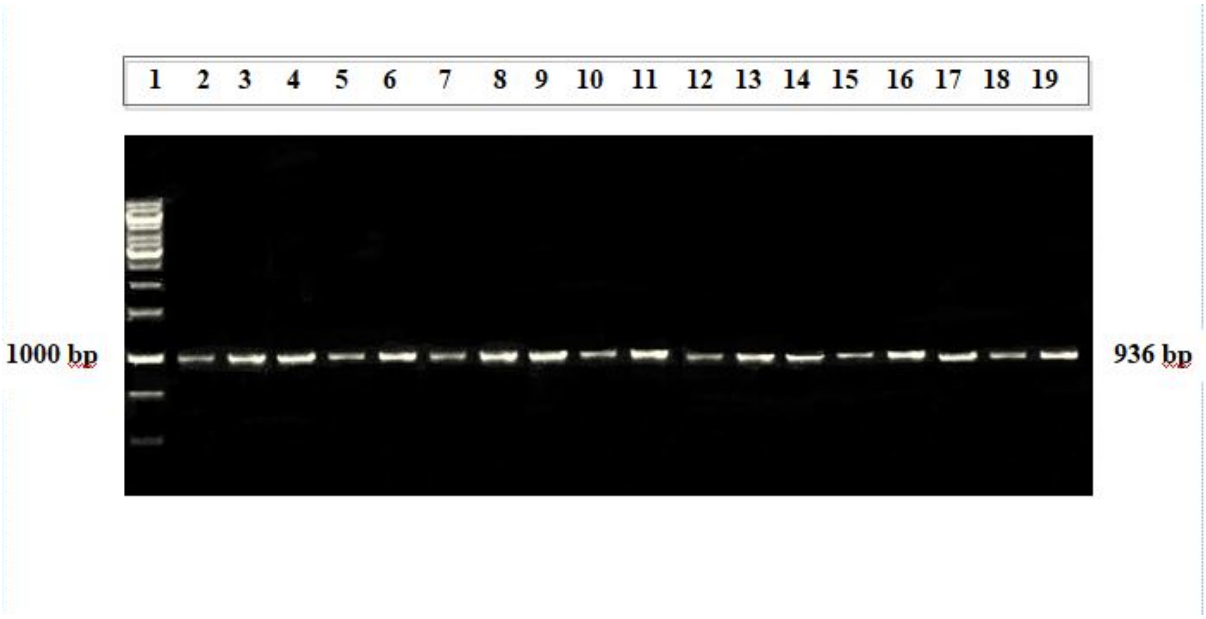

Fig. 1: PCR amplification of *vanA* gene of approximately 936 bp. Lane 1 shows 1 kb ladder while lane 2 – 19 shows vancomycin resistant *E. faecium* and *E. faecalis*

3.2 Relationship of VRE and VSE bacteraemia with age, gender and source

Age and source had significant impact on VRE and VSE bacteraemia occurrence while gender had no such impact (Table 1, Fig. 2). VRE was found significantly more prevalent in age group of over 50 years (p = 0.002) whereas VSE was observed in both age groups albeit with higher occurrence in below 50 group. Similarly VSE was mostly isolated from urine samples with few from blood as compared to VRE that was more frequent in blood (p =0.000). Gender analysis showed revealed both VSE and VRE were more in males than females, albeit with no statistical significance (p> 0.05).

Table 1: Frequency distribution of VRE and VSE bacteraemia related to age, gender and source and significance of each variable on VRE and VSE occurrence.

Variables	VRE	VSE	Frequency (N=49)	Percentage	*p*-value
Age					
Below 50	5	23	28	57.1	0.002
Above 50	13	8	21	42.9	
Source					
Blood	10	2	12	24.5	
Urine	8	25	33	67.3	0.000
Throat	0	4	4	8.2	
Gender					
Male	15	22	37	75.5	0.332
Female	3	9	12	24.5	

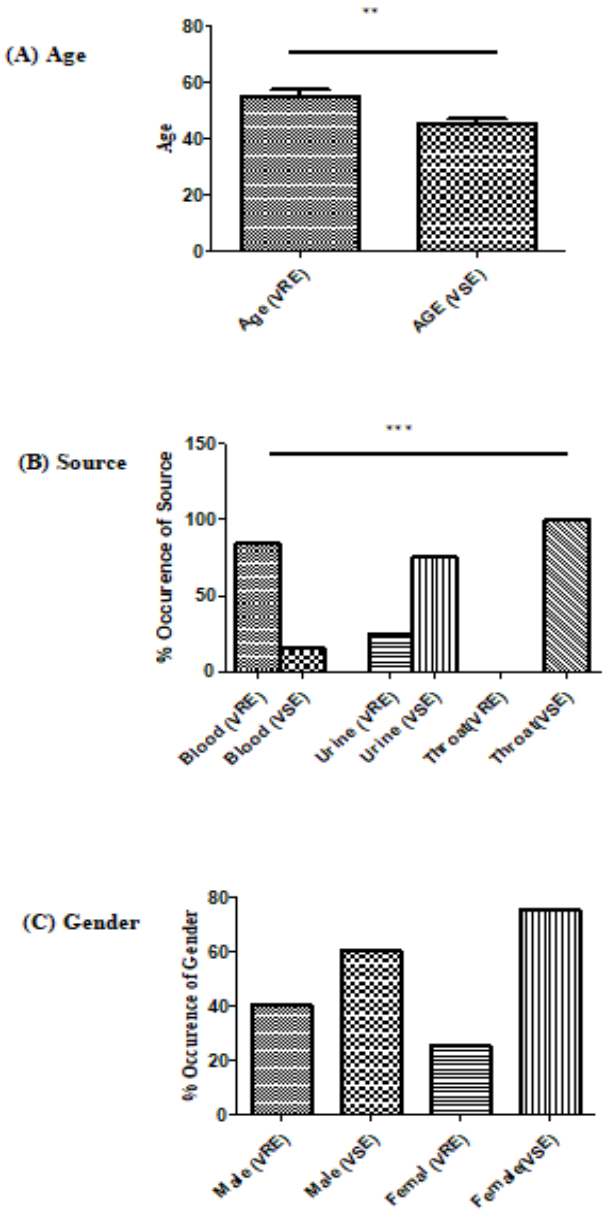

Fig. 2: VRE and VSE bacteraemia occurrence on the basis of different **(A)** age groups **(B)** sample sources and **(C)** gender.

4. DISCUSSION

E. faecalis and *E. faecium* are notorious for hospital acquired infections across the world, *E faecium* being the most important because of its high resistance to antimicrobials and high mortality rate[1,13]. Resistance against a number of commonly used antibiotics like beta-lactams, glycopeptides and aminoglycosides is the most important feature of both *E. faecalis* and *E. faecium*[8]. Medical community acknowledges the ever increasing resistance against vancomycin and its spread to other species, and consequent emergence of multi-drug resistant microbes as a huge problem[14]. The determinants responsible for vancomycin resistance in *Enterococci* are predominantly *vanA* and *vanB; vanA* being common among most geographic locations[15].

Treatment options for VRE include drugs like tigecycline, linezolid, daptomycin, quinipristin-dalfopristin, platensimycin, nitrofurantoin and fosfomycin. However, some reports of resistance are also documented against these as well[16]. These organisms have become a challenge for physicians due to their high mortality and morbidity rate[17].

In this study 49 clinical isolates of *E. faecium* and *E. faecalis* obtained from various hospitals of Islamabad and Rawalpindi revealed that vancomycin resistance might be rapidly spreading in this part of Pakistan. Among 49 strains, 18 were phenotypically resistant to vancomycin (data not sown) while others were susceptible for it. All strains were subjected to PCR amplification for *vanA* and *vanB* gene cluster. Phenotypically resistant strains were positive for *vanA* while no strain was positive for *vanB*. Bands of approximately 936 bp were obtained for *vanA* gene which is in accordance with a previous report[18]. The localization of *vanA* gene on plasmid DNA was also in accordance with published data[19].

The possible explanation of *vanB* absence may be attributed to difference in the prevalence of these genes in the study area, a finding in line with previously published reports from India[15]. Although *vanB* gene has been reported in Spanish, English and American isolates, it was far less than *vanA*[20-21]. Studies from Korea and Taiwan have, however reported *vanB* presence on a higher level[22-23]. Our findings were in conformance with *vanA* prevalence in neighbouring India so it can be suggested that geographic distribution leads to the prevalence of *vanA* gene in Pakistani population.

The statistical analysis revealed two dependant factors being age and source for bacteraemia due to both VRE and VSE. Gender emerged as an independent factor for both cases, albeit with increased proportion for male patients. A possible explanation for higher age dependence in VRE bacteraemia may lie in the fact that Entercoccal Surface Protein (ESP) causes immune evasion in elderly patients who already have a compromised immunity. Increased exposure to the antibiotics in elderly patients might have contributed to this phenomenon.

Frequent isolation of VRE from blood samples may be attributed to the opportunistic nature of organism and antibiotic pressure or other environmental factors that are known for transforming enterococcal normal flora to potential pathogens[13].

5. CONCLUSIONS

It can be concluded from this study that irrational use of vancomycin and other antibiotics, and poor clinical practice might have contributed to increase prevalence of VRE. This study reports *vanA* gene cluster responsible for resistance in Pakistani population that warrants effective intervention strategies to inhibit VRE.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Willems RJ, van Schaik W. Transition of Enterococcus faecium from commensal organism to nosocomial pathogen. Future Microbiol. 2009;4(9):1125-35.
2. Sreeja S, Babu PRS, Prathab AG. The prevalence and the characterization of the enterococcus species from various clinical samples in a tertiary care hospital. J Clin Diagn Res. 2012;6(9):1486-8.

3. Lebreton F, Willems RJL, Gilmore MS. Enterococcus Diversity, Origins in Nature, and Gut Colonization. 2014 Feb 2. In: Gilmore MS, Clewell DB, Ike Y, et al., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK190427/ Accessed on 25 September 2020
4. Peel T, Cheng AC, Spelman T, Huysmans M, Spelman D. Differing risk factors for vancomycin-resistant and vancomycin-sensitive enterococcal bacteraemia. Clin Microbiol Infect. 2012;18(4):388-94.
5. Zhang Y, Du M, Chang Y, Chen L, Zhang Q. 2017. Incidence, clinical characteristics, and outcomes of nosocomial Enterococcus spp. bloodstream infections in a tertiary-care hospital in Beijing, China: a four-year retrospective study. Antimicrob Resist Infect Control. 2017;6:73
6. Marcone GL, Marinelli F. Glycopeptides: An Old but Up-to-Date Successful Antibiotic Class. Antimicrobials. 2013; 85-107
7. Hawkes N. Modifications to vancomycin raise hope for combating antibiotic resistance. BMJ. 2017; 357:j2661
8. Mundy LM, Sahm DF, Gilmore M. Relationships between enterococcal virulence and antimicrobial resistance. Clin Microbiol Rev. 2000;13(4):513-22.
9. Lester CH, Frimodt-Moller N, Sorensen TL, Monnet DL, Hammerum AM. In vivo transfer of the vanA resistance gene from an Enterococcus faecium isolate of animal origin to an E. faecium isolate of human origin in the intestines of human volunteers. Antimicrob Agents Chemother. 2006;50(2):596-99.
10. Protonotariou E, Dimitroulia E, Pournaras S, Pitiriga V, Sofianou D, Tsakris A. Trends in antimicrobial resistance of clinical isolates of Enterococcus faecalis and Enterococcus faecium in Greece between 2002 and 2007. J Hosp Infect. 2010;75(3):225-7.
11. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015; 8:217–230
12. Taylor ME, Oppenheim BA, Chadwick PR, Weston D, Palepou MF, Woodford N, et al. Detection of glycopeptide-resistant enterococci in routine diagnostic faeces specimens. J Hosp Infect. 1999;43(1):25-32.
13. Conde-Estevez D, Grau S, Albanell J, Terradas R, Salvado M, Knobel H. Clinical characteristics and outcomes of patients with vancomycin-susceptible Enterococcus faecalis and Enterococcus faecium bacteraemia in cancer patients. Eur J Clin Microbiol Infect Dis. 2011;30(1):103-8.
14. Arias CA, Contreras GA, Murray BE. Management of Multi-Drug Resistant Enterococcal Infections. Clin Microbiol Infect. 2010;16(6):555-62.
15. Sood S, Malhotra M, Das BK, Kapil A. Enterococcal infections & antimicrobial resistance. Indian J Med Res. 2008;128(2):111-21.
16. Isenman H, Fisher D. Advances in prevention and treatment of vancomycin-resistant Enterococcus infection. Curr Opin Infect Dis. 2016;29(6):577–582
17. Hayakawa K, Marchaim D, Martin ET, Tiwari N, Yousuf A, Sunkara B, et al. Comparison of the clinical characteristics and outcomes associated with vancomycin-resistant Enterococcus faecalis and vancomycin-resistant E. faecium bacteremia. Antimicrob Agents Chemother. 2012;56(5):2452-8.
18. Miele A, Bandera M, Goldstein BP. Use of primers selective for vancomycin resistance genes to determine van genotype in enterococci and to study gene organization in VanA isolates. Antimicrob Agents Chemother. 1995;39(8):1772-8.
19. Arthur M, Reynolds P, Courvalin P. Glycopeptide resistance in enterococci. Trends Microbiol. 1996;4(10):401-7.
20. Gold HS, Unal S, Cercenado E, Thauvin-Eliopoulos C, Eliopoulos GM, Wennersten CB, et al. A gene conferring resistance to vancomycin but not teicoplanin in isolates of Enterococcus faecalis and Enterococcus faecium demonstrates homology with vanB, vanA, and vanC genes of enterococci. Antimicrob Agents Chemother. 1993;37(8):1604-9.
21. Gold HS. Vancomycin-resistant enterococci: mechanisms and clinical observations. Clin Infect Dis. 2001;33(2):210-9.
22. Yang J, Lee D, Kim Y, Kang B, Kim K, Ha N. Occurrence of the van genes in Enterococcus faecalis and Enterococcus faecium from clinical isolates in Korea. Arch Pharm Res. 2007;30(3):329-36.
23. Lu JJ, Chang TY, Perng CL, Lee SY. The vanB2 gene cluster of the majority of vancomycin-resistant Enterococcus faecium isolates from Taiwan is associated with the pbp5 gene and is carried by Tn5382 containing a novel insertion sequence. Antimicrob Agents Chemother. 2005;49(9):3937-9.