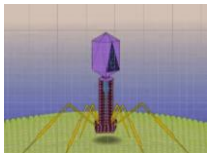

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Identification of Vancomycin Resistant Enterococcus Genes in Clinical Isolates

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ABSTRACT: *Vancomycin resistant enterococci (VRE) nosocomial diseases are rapidly spreading across the world. To treat these diseases is becoming a great challenge due to antibiotic resistance. The present study was aimed to estimate the prevalence of vancomycin resistant enterococci and to find out genes responsible for causing vancomycin resistance. One hundred different blood, wound and urine samples were collected from hospitalized patients at tertiary care health hospital, Lahore. On the basis of colony morphology, isolates of Enterococci were identified, Gram staining and biochemical tests including catalase test, Bile esculin test, growth in 6.5% NaCl, litmus milk reduction were performed. All the clinical isolates were checked for vancomycin resistant and 15% of all the samples were vancomycin resistant. Polymerase chain reaction (PCR) was performed for vancomycin resistance, enterococcal isolates to identify van genes responsible for resistance. In our study the presence of vanA gene was found in all 15 vancomycin resistant samples. vanB gene was not detected in any of the sample. The study highlights the increased VRE prevalence in clinical isolates obtained from local hospital. The presence of vanA gene in all resistant samples is suggestive of its role as resistant gene. On the basis of these results there is an urgent need of establishing a rational antibiotic use policy for better management of enterococcal infections.*

Keyword: *Antimicrobial resistance, E. faecalis, vancomycin, PCR*

INTRODUCTION

Bacterial resistance to antimicrobial agents has tremendously increased around the globe over the past few years (Livermore, 2009). *Enterococcus* and *Staphylococcus aureus* are the gram-positive organisms with the high potential of carrying resistance and clinical

impact (Theuretzbacher, 2013). The *Enterococci* are vigorous and adaptable species capable of surviving under harsh conditions can individually exist as chains, pairs, or groups which make them well adapted to the environment of health care facility and are also known as ovoid bacteria with non-spore forming properties. Multidrug resistance is very common with this bacterium ultimately causing an increase in treatment cost, prolonged hospital stays, risk of treatment failure and mortality therefore rising a public health threat (García-Solache and Rice, 2019). They can hydrolyze esculin where there is pigment of bile, can live at 60°C for 30 minutes and can grow at both 10-45°C, pH 9.6, and sodium chloride (NaCl) 6.5% (Babar et al., 2014). *Enterococci* are usually a component of the common human flora of feaces. In hospitalized patients, sensitive delicate soft tissue wounds, ulcers, and the gastrointestinal tract are the main sites of colonization (Shete et al., 2019).

Virulence factors are more apparent in *E. faecalis*, elucidating that still it has major role in infections of *Enterococci* (Vu and Carvalho, 2011) Antibiotics are very useful agents to treat bacterial infections. Evidence of treating infections is present in Greece, Egypt and China in ancient times but the modern era of treating infections started with the discovery of penicillin. Vancomycin was introduced for treatment in 1970, s for the treatment of MRSA, but in early 1980s its resistance was reported that was a major threat in the medical field. There are many reasons that contributes to the resistance development like overuse of drug, improper prescription and fewer number of new antimicrobial agent development and availability (Ventola, 2015). Vancomycin is derived from Streptococcus orientalis. Vancomycin is structurally unrelated to any currently available antibiotic this property makes it unique. It also has a unique mode of action. Bactericidal effect of vancomycin is mediated by inhibiting the bacterial cell wall polymerization of peptidoglycans and for this purpose uridine diphosphate – N – acetylmuramyl (UDP-Mur-NAc) penta peptide acts as substrate and then peptidoglycan synthesis is inhibited by vancomycin during membrane preparations. This tricyclic glycopeptide antibiotic is intended for the prevention and treatment of a range of infections caused by gram positive bacteria, includes *Streptococcus pneumonia*, *S. aureus*, *S. pyogenes*, *S. epidermidis*, *S. bovis*, *S. agalactiae*, *S. mutans*, *Clostridium* species, *Listeria monocytogenes*, *Lactobacillus* species & *diphtheroids*. With the combination of an aminoglycoside, antimicrobial

activity of vancomycin is enhanced against different pathogens like *Enterococci, S. bovis* and *S. aureus* (Marsot et al., 2012).

Recent years provide details regarding vancomycin resistant enterococci (VRE) nosocomial diseases have quickly expanded in numerous countries around the world, and this microorganism is now being documented for clinical significance (Vilela et al., 2006). Five genes are known to be involved and are considered accountable for resistant to antibiotics (glycopeptides) in strains of vancomycin resistant *Enterococci* (VRE). Two genes (vanA and vanB) are known to be most common than remaining, particularly in *Enterococcus faecium* and *Enterococcus faecalis*. vanB genes strains have shown resistance to vancomycin and sensitivity to tycoplanin, on the other hand vanA containing strains are resistant to both vancomycin and tycoplanin. Vancomycin resistant Enterococci strains are considered to be transmitted by fecal carrier hands of health care practitioner. ICU patients, critically ill children and elders are more likely to get infected (Honarm et al., 2012). vanA genotype strains are distinguished by high resistance level of teicoplanin and vancomycin while, strain of vanB shows medium to higher resistance for vancomycin (Cetinkaya et al., 2000). The present study aimed to find genes that responsible for causing vancomycin resistance.

MATERIAL AND METHODS

Isolation and Identification of Bacterial strain

A total of 80 clinical samples including blood, wound, urine and stool samples were taken from the patients of Lahore Health Care Hospital. Samples were kept at 4°C until further investigation. Gram staining and biochemical tests including such as catalase, bile esculin test, growth in 6.5% NaCl, Lactose ferment test (also ferment mannitol), Lancefield grouping, litmus milk reduction test, CAMP reaction, and pigment production were performed on bacterial isolates and indicated the presence of *E. faecalis* (Hansen et al., 2004).

McFarland density standards were employed to standardize the susceptibility of test inoculums (Hudzicki, 2009). The antibiotic assay in this research article was conducted using the methodology described by Postek in 2022.

Molecular identification of *E. faecalis* using PCR

For molecular identification, DNA of bacterial samples was isolated and amplified by PCR as described by Wang et al., 2011. Following DNA

extraction, isolates were subjected to PCR for the identification of the vanA and vanB genes. In the PCR tube, 8.5µL of nuclease-free water was added. After that, 12.5µL of master mix was added. The identical combination received 2µL of extracted DNA. Finally, 1µL of forward primer and 1µL of reverse primer were added to the tube, giving it a total capacity of 25l. The PCR tubes were then placed in the thermal cycler (PTC 06 ICCC, Pakistan) to begin the PCR reaction. The PCR bands were then visualized in doc illuminator (Meradd-ICCC).

RESULTS

Enterococci were isolated from clinical samples (Blood, wound, stool and urine). The samples were identified on the basis of colony morphology, gram staining and biochemical testing (Catalase, litmus milk decolorization, Bile Aesculin Hydrolysis tests).

Antibiotic Susceptibility Test

Vancomycin-resistant Enterococci positive isolates were detected at the molecular level. WizPrTM gDNA Mini Kit (cell/tissue) was used to extract DNA from isolates.

Detection of vanA and vanB gene The DNA was extracted using the standard procedure WizPrTM gDNA Mini Kit (cell/tissue). The polymerase chain reaction mixture and protocols for amplification of vanA and vanB were followed. PCR products were analyzed by electrophoresis. A clear band of vanA gene was found and shown in figure 1. PCR result was negative for all samples for *van*B gene.

Antimicrobial sensitivity results

While, the percentages of resistant and sensitive of *E. faecalis* isolates against different antibiotics have been noticed and reported in Table 1.

Table 1. **Bacterial isolates exhibit distinct antibiotic sensitivity and resistance patterns**

Antibiotics	Sensitivity %age	Resistance %age
Vancomycin (VAN)	85 %	**15 %**
Ciprofloxacin (CIP)	58%	**42%**
Gentamycin (GEN)	39%	**61%**
Streptomycin (STP)	45%	**55%**
Ampicillin (AMP)	**80%**	**20%**

Identification of VRE Resistant Genes

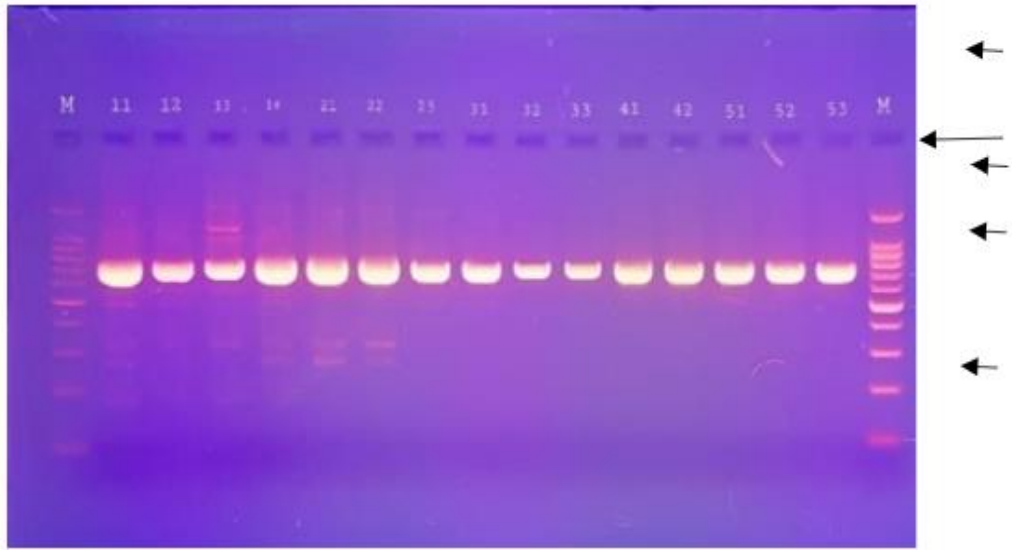

Fig. 1. Gel Electrophoresis Analysis of PCR Product for *van*A gene.

Table 3. Sequence of Primer *van*A and *van*B

Sr. No.	Gene	Primer (5'-3')	Size of product (bp)	References
1	*Van*A	F-GGGAAAACGACAATT R-TACAATGCGGCCGTTA	732	**Reviriego** et al**. 2005**
2	*Van*B	F-ACGGAATGGGAAGCCGA R-TGCACCCGATTTCGTTC	647	**Reviriego** et al**. 2005**

DISCUSSION

VRE was first identified as hospital-associated pathogens in Europe in the late 1980s, and afterwards was found to be disseminated worldwide. Reportedly depending on the site of infection, the resistance rates to National Healthcare Safety Network during 2009 between 6.2% and 9.8% for *E. faecalis* 62.3% and 82.6% for *Enterococcus faecium* and VRE resistance remained low in Europe as the reported rates were 19.0% to 5.5% for *E. faecalis* and *E. faecium* in hospital settings, respectively (European Centre for Disease Prevention and Control)

Annual Epidemiological Report 2013, reporting on 2011 surveillance data and 2012 epidemic intelligence. Multidrug resistance is very common with this bacterium ultimately causing an increase in treatment cost, prolonged hospital stays, risk of treatment failure and mortality therefore rising a public health threat (García-Solache and Rice, 2019). Numerous traits in both species have known to have high linkage with disease causing potential. These consist of the ability to evade the immune system, host cells attachment competency, capacity to stick on to a variety of extracellular protein lattice after that colonization and attack into the host tissues lead to adjustment of the host insusceptibility and creation of pathological alterations clearly by toxin and enzyme production through inflammation induction, contributing to pathogenesis and severity of Enterococci contamination (Courvalin, 2006).

A study carried out by (Ghoshal et al., 2006) shows that out of 685, *E. faecalis* were 456 and E. faecium were 229 in number. In 10 cases resistance was confirmed about 1.4%. (Correia et al., 2016) stated VRE prevalence of about 3.3% (Aleyasin et al., 2007) demonstrated 9.5% of VRE prevalence. This gradual increase in the frequency of VRE in our population is alarming and its major cause is impulsive and excessive vancomycin usage. All the clinical isolates were found to be vancomycin resistant. In our study the presence of vanA gene was found in all of 15 samples out of 15. The presence of vanB gene was not found in any of our screened samples as an Indian study showed VRE about 12% and VRE isolates carried 100% vanA gene (Shete et al., 2019) in another study vanA gene was dominant about 79 % and remaining 20% was vanB gene (Protonotariou et al., 2010). An investigation done by (Kuriyama et al., 2003) vanA were found to be dominant gene responsible for resistance in Enterococci. (Aleyasin et al., 2007) demonstrated in an investigation that vanA phenotype is responsible for almost 70%.

In a study done by (Sreeja et al., 2012) the prevalence rate of Enterococcus was found out to be 2.3%. Similarly (Moosavian et al., 2021) stated that the multiplex PCR assay showed that all isolates of Enterococcus had vanA gene, and vanB was not found in any of screened isolates.

In study, out of total 53 *E. faecalis* isolates, resistance was found in 43% strain. vanA gene was present in 37.7% of isolates (Taji et al., 2019). In research out of 160 isolates, 125 were *E. faecalis* and 35 were *E. faecium*. Twenty-seven isolates were vanA gene carrier and none of them carried vanB

gene. *E. faecalis* carried increased capacity of resistance (Jahansepas et al., 2018). Another investigation revealed that out of 300 specimens 20% were found to be Enterococci, and *E. faecium, E. faecalis* and other *Enterococci* were 53.3%, 31.7% and 10% respectively. vanA gene was found in 47.4% of isolates, while 33.3% of VRE had vanB gene (Khairy et al., 2019).

The complete epidemiology of VRE has not been explained yet. However, it is known that certain patient populations such as critically ill patients aged or immunosuppressive such as ICU patients and those who have had an extended hospital stay or received multiple antimicrobial agents are at increased risk for VRE infection. There are limited therapeutic alternatives so Treatment of infections caused by VRE is enormously challenging. While treating VRE infections an alarming problem is emergence linezolid resistance, even though linezolid has demonstrated well anti enterococcal activity.

CONCLUSION

Vancomycin-Resistant Enterococcus (VRE) were identified in the samples and vanA was the major resistance gene. The findings highlighted the vital importance of sensible antibiotic policy to effectively control enterococcal infections. To prevent the spread of VRE, effective infection control measures must be implemented.

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