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Original Research Article

Prospective review of Vancomycin Resistance among Enterococcus faecalis in a tertiary care teaching hospital.

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Abstract:

Introduction: Vancomycin stays remains the drug of choice for resistant gram-positive diseases brought about by Enterococcus spp. Increased use of vancomycin has led to frank resistance and increase in MIC (MIC creep). Vancomycin-resistant Enterococci (VRE) are important emerging nosocomial pathogens resulting in treatment failures.

Aim: This study was undertaken in view to detect resistance to vancomycin among clinical isolates of Enterococcus faecalis by phenotypic and genotypic methods.

Materials and Methods: A cross sectional study was conducted in a teaching hospital from January 2020 to July 2021. In this study we have included only non-repetitive, consecutive clinically significant Enterococcus faecalis (124). They were identified up to species level by conventional methods. Susceptibility to various antibiotics was tested by disc diffusion method. MIC of vancomycin was determined by agar dilution method. All 124 isolates were subjected to polymerase chain reaction (PCR) to detect van A and van B genes in this study.

Results: Out of 124 Among *Enterococcus faecalis*, twenty-one (16.9%) and seven isolates (5.6%) exhibited resistance to vancomycin and teicoplanin by disc diffusion respectively. All isolates were susceptible to linezolid. Van A was detected in three, van B in eight and two had both van A and van B.

Conclusion: PCR remains the gold standard for determination of vancomycin resistance. Thirteen isolates (10.4%) of *Enterococci* were vancomycin-resistant.

Keywords: *Enterococci*, van A, van B, Enterococci, VRE

Introduction

Even though Enterococci are members of the healthy human intestinal flora, causing several infections in human and are also leading causes of highly antibiotic-resistant, hospital-acquired infection [1]. There is developing proof that these microorganisms much of the time possess several specific traits that empower them to make survive in the hospital environment, colonize patients, and cause infections such as bacteraemia, peritonitis, endocarditis and urinary tract, surgical site infection, and device-related infections [2]. The genus Enterococcus is of increasing significance as a cause of nosocomial infections, and this trend is exacerbated by the development of antibiotic resistance [3]. Therapeutic spectrum of enterococci is limited since the organisms are genetically resistant to Cephalosporins and Cotrimoxazole. They also have a tremendous capacity to acquire resistance to penicillins, high concentration of aminoglycoside & vancomycin [4]. Enterococci with High-Level Resistance to Aminoglycosides (HLAR), beta lactamase production & glycopeptide resistance including vancomycin resistance are posing a great therapeutic challenge for clinicians as well as health care institutions [5].

Antimicrobial resistance results in increased morbidity, mortality and costs of treatment. Preventing the emergence and dissemination of resistant organisms is critical for control of hospital infections. Appropriate antimicrobial stewardship that includes optimal selection, dose and duration of treatment, as well as control of antibiotic use, will prevent or slow the emergence of resistance among microorganisms [5]. Cell wall synthesis inhibiting

Glycopeptide antibiotics remain the drug of choice for infections caused by resistant *Enterococcus s*pecies. If Enterococci shows with a MIC of ≥32µg/ml are classified as vancomycin resistant *Enterococci (*VRE). Vancomycin resistant *Enterococci* have emerged as important nosocomial pathogens in the last two decades throughout the world [6]. VRE are associated with many infections ranging from mild to life threatening. Extensive use of vancomycin to treat infections with MRSA has led to decreased susceptibility to vancomycin among *Enterococci*. As of today, very limited options are available for treating serious infections caused by VRE [6]. VRE are important nosocomial emerging pathogens resulting in treatment failures. This study was undertaken to detect vancomycin resistance among *Enterococcus faecalis* isolates by both phenotypic and genotypic methods.

Materials and Methods

Study Isolates

The study was conducted in tertiary care hospital from January 2020 to July 2021. Sample was collected after getting approval from Institutional ethics committee. The exclusive criteria included the isolates which were not clinically significant and those who are already on antibiotics.

Blood, Urine, Pus, wound swab was included in this study. Pus culture was done in MacConkey and Blood agar base. Mid-stream urine was collected and cultured on Blood agar and MacConkey agar, and CLED. All the inoculated media are incubated overnight at 37°C and suspected colonies was picked up and subjected to identification by biochemical tests. For identification we have done catalase, and Bile-esculin test is based on the ability of certain bacteria, notably the group D streptococci and Enterococcus species, to hydrolyze esculin in the presence of bile (4% bile salts or 40% bile).

Antibiotic Susceptibility

Disc Diffusion

Antibiotic susceptibility was tested for *Enterococcus faecalis,* with gentamycin (120µg), ciprofloxacin (5µg), vancomycin (30µg), teicoplanin (30µg) and linezolid discs (30µg) for all isolates, erythromycin (10µg) for exudative isolates, nitrofurantoin (300µg) for urinary isolates by disc diffusion method [7]. All the discs were procured from Hi-media laboratories, Mumbai, Maharashtra, India.

Minimum inhibitory concentration

MIC of vancomycin for all the test isolates was determined by agar dilution method; the range tested being 0.008µg/ml to 256µg/ml in accordance to CLSI guidelines [7].

Molecular Detection of Genes

All the isolates were subjected to PCR which targeting van A and van B. A single isolated colony was taken and inoculated in Luria-Bertini broth further incubated for 20 hours with intermittent shaking in between and from this 1.5 ml of was centrifuged for about 5 minutes. The pellets were suspended in 500 µl of distilled water and then lysed by heating at 95°C for 5 minutes and again centrifuged for 1 minute. 5 µl of this extract was used as a template for amplification. The following is the list of primers used [Table-1].

PCR Conditions: Initial denaturation - 95 oC - 3 min

Denaturation: 94 oC - 1 min

Annealing: 56 oC - 1 min (van A & van B)

Extension: 72 oC - 1 min

Final Extension: 72 oC - 5 min

PCR Product: PCR Product of 782 bp (van A) & 297 bp (van B) were visualised by Agarose gel electrophoresis.

Table 1: Primer sequence for van A, van B

Primer	Primer sequence (5'–3')	Product size	Annealing Temperature
van A	P1 = GCT ATTCAG CTG TAC TC P2 = CAG CGG CCA TCA TAC GG	783 bp	56ºc
van B	P1 = CAT CGC CGT CCC CGA ATT TCA AA P2 = GAT GCG GAA GAT ACC GTC GCT	297 bp	56ºc

Results

Bacterial isolates of *Enterococcus faecalis* (124) were included in this study. The *Enterococcus faecalis* were collected from urine (57) and exudative (67) samples.

Disc Diffusion *(Enterococcus faecalis)*

The resistance to high level gentamycin, ciprofloxacin, erythromycin and nitrofurantoin were 48.6%, 83.03%, 86.19% and 48.18% respectively. Vancomycin and teicoplanin resistance were exhibited in 16.9% (21) and 5.6% (7) isolates respectively. All isolates were susceptible to linezolid.

Minimal Inhibitory Concentration

Enterococcus Faecalis

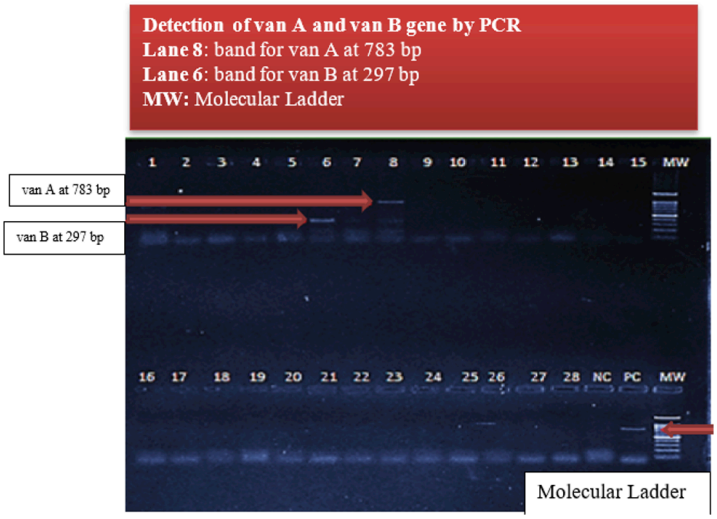
MIC range has been set between 0.25-256 µg/ml. MIC_{50} was 1 µg/ml. Thirteen isolates were resistant to vancomycin. Seven of them had MIC of >256µg/ml, three with a MIC of 256µg/ml and two with a MIC of 128µg/ml. Four were in the

intermediate range with a MIC of 8 µg/ ml and 16µg/ml.

Polymerase Chain Reaction

Enterococcus faecalis

Three isolates harboured van A and eight isolates harboured van B. In two isolate both van A and van B genes were detected [Fig-1].



Among *Enterococcus faecalis* Correlation of results between MIC and PCR, out of thirteen, seven isolates which were resistant by MIC, three isolate (MIC>256µg/ml) was positive for van A. Other four were neither van A nor van B positive. Out of three isolates with van A, two had MIC in resistant range (256µg/ml), other with MIC in intermediate range (16µg/ml). All eight isolates with van B had MIC in a susceptible range (1µg/ml). The isolate with both van A and van B had a MIC in intermediate range (8 µg/ml).

Discussion

Enterococci are commensal microbes occupying the digestion tracts of the both humans and animals, which are the major restrictively pathogenic microorganisms that cause hospital-acquired infections. As of late, successive unseemly utilization of antimicrobial agents, With the antimicrobial agents being frequently used in clinical treatment, antibiotic-resistant enterococci, particularly multi-drug resistant enterococci isolates, such as vancomycin-resistant enterococci (VRE) and linezolid resistant enterococci (LRE) have emergence and spread all over the world [8,9]. Likewise, the emergence of High-Level Aminoglycoside-Resistant (HLAR) enterococci and Vancomycin-Resistant Enterococci (VRE) causes incredible troubles in clinical anti-infective therapy [10-12]. *Enterococci*, aside from being a part of normal microbiota additionally cause nosocomial infections. Vancomycin is a cell wall acting glycopeptide discovered as early as 1950. It acts by preventing synthesis of peptidoglycan precursors of cell wall by blocking transglycosylation and transpeptidation which is the steps essential for cross linking. Site of action is on D-ala D-ala residue of the polypeptide [13]. Vancomycin has been used as the drug of choice in serious infections caused by resistant *Enterococci.* Resistance of these organisms to vancomycin is being reported since last two decades. The first VRE was reported by Uttley et al., in 1977 from Great Britain [14]. In India, the first VRE was reported by Mathur et al., in 1999 [15]. In this review we have included 124 *Enterococcus faecalis* isolates in exudates were 67(54%) and 57 (46%) from urine. The resistance to high level gentamycin, ciprofloxacin, erythromycin and nitrofurantoin were 48.6%, 83.03%, 86.19% and 48.18% respectively. Another study reported resistance of 37%, 74.38% and 29% to high level gentamycin, ciprofloxacin and nitrofurantoin among Enterococci [16]. Also, it has been well notable that resistance to vancomycin in *Enterococci* is mediated by van genes. To date, van A, van B, van C, van D, van E, van G, van L, van M, and van N have been identified [17]. Van A and van B genotypes have predominated reported worldwide [18]. Van A is associated with high level resistance to both vancomycin (MIC≥64µg/ml) as well as teicoplanin (MIC≥16µg/ml). In other hand Van B is associated with varying levels of resistance to vancomycin alone (MIC 4 -1000 µg/ml) with susceptibility to teicoplanin [19]. In our study, by MIC determination thirteen isolates were vancomycin resistant and four isolate exhibited intermediate susceptibility to vancomycin. The three isolate that harboured the van A gene had a MIC of 256µg/ml. The other four neither carried van A nor van B. They have to be screened for other van genes (van D, van E, van J, van L, van M) for further characterization to identify the vancomycin resistant [6]. With PCR finding, three isolates were van A Positive. One had a MIC of 256µg/ml. Other two had a MIC of 16 µg/ml

showing low level resistance to vancomycin. Such van A genotype –van B phenotype incongruency has additionally been reported in other reviews in *Enterococcus faecalis* and *Enterococcus faecium* [20]. Park et *al.,* suggested this could be due to presence of insertion sequence IS 1216v in coding region of van S gene as a probable reason for this incongruency [20]. In other hand some authors this is due to mutations in van A gene cluster or in van S regulatory element [21]. Eight isolates were positive for van B. All the isolates had a susceptible MIC range was 1 µg/ml. well known fact that Van B VRE with susceptible MIC were already reported in few studies in *Enterococcus faecium* [22]. The reason for this phenotype-genotype incongruence is not known. Two isolate which carried both van A and van B had an MIC of 8µg/ml. in this study 10.5% of all *Enterococcus* isolates are VRE proven by PCR. Another study which conducted in South India reported 8.7% VRE [14,23]. van B was the most common phenotype. This is in contradiction to many studies which report van A as the commonest phenotype [24]. Our study has several limitations. Firstly, this is a short period and sample size small in this study, not all the VRE isolates could be collected for the further analysis; Secondly, this is a single centre study, our data may not reflect all the characterization of VRE isolates from other institutions in Puducherry, since the burden of VRE has been shown to vary regionally.

Conclusion

Among *Enterococcus faecalis* isolates, 10.5% were VRE by molecular PCR method. High percentage of resistance to different antibiotics agents among *Enterococcus faecalis* isolates was additionally recorded in this review. van A genotype –van B phenotype incongruence was observed in three of the test isolates. Another important finding is VRE isolates with susceptible MIC. van B was the commonest genotype prevalent in *Enterococcus spp*. Even now PCR remains the gold standard method for diagnosis of vancomycin resistance *Enterococcus*. Emerging vancomycin resistance among *Enterococcus faecalis* is a cause for concern as this leads to a great difficulty in treating the serious infections caused by them. Accordingly, our findings indicated the significance of performing MIC and PCR for detection of genes for antibiotic resistance which will help to do surveillance for infection control practices. Prudent use of antibiotics with good infection control practices will help to retain their susceptibility.

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