

Prevalence of VanA and B Genotype Among Vancomycin Low Resistant Enterococcus in Fecal Normal Flora and Clinical Samples Isolated From Tehran's Hospitals

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Background: Vancomycin-Resistant Enterococci (VRE) pose an emerging problem in Iran hospitals.

Objectives: The present study was undertaken to determine the prevalence of van genes among vancomycin low resistant Enterococcus (VLRE) isolate in Tehran hospitals.

Materials and Methods: Totally 162 and 152 isolates of enterococcal species were obtained from fecal and clinical samples of hospitalized patients between March and November of 2012. The antibiotic susceptibility of the isolates and minimum inhibitory concentration (MIC) were determined according to CLSI. The presence of the van A and B resistant genes in VLREs isolates were evaluated by PCR method.

Results: VLRE accounted for 162 (38.4%) and 159 (38.7%) of Enterococcal isolates from clinical and fecal flora samples respectively (MIC's in the range of 16 to 64 µg/mL). VanA and vanB genotypes were detected with polymerase chain reaction (PCR) in 85 (27%) and 35 (11%) of VLRE isolates, respectively.

Conclusions: VLRE cause serious problems in healthcare settings because their detection is difficult and treatment of these infections may not be successful. These species are miss-identified as vancomycin susceptible isolates. By detection of VLRE, we can evaluate perspective of vancomycin high level resistant Enterococcus rate in future.

Keywords: Polymerase Chain Reaction (PCR); Enterococcus; VanA protein; VanB protein

1. Background

Surveillance for VRE, which have appeared as an important of enteric pathogens during the past years in the world, is becoming an important aspect of infection control management within hospitals especially intensive care unit (ICU) (1).

Although Enterococcus is an intestinal micro flora, this gram positive coccus causes serious infections in human (1). Enterococcal infections usually occur in hospitalized patients with serious underlying illnesses such as cancer, blood disorders, kidney disease and immune deficiencies (2-5). Healthy people are not at risk of infection, but health care workers could play a role to transmit the organism if careful hand washing and other infection control precautions are not practiced (6, 7).

Enterococci exhibit intrinsic resistance to several antibiotics and have an ability to acquire antibiotic resistance rapidly (8). In the recent years, incidence of glycopeptides resistant Enterococcus in hospitalized

patients has been increased rapidly (9). Vancomycin-resistant enterococcal infections in Iran, like many other countries, have been associated with high morbidity and mortality rates especially in immune-compromised patients (10-12).

2. Objectives

The aim of this study was to determine the prevalence of van genes among the VLRE in clinical and fecal normal flora samples from five hospitals in Tehran.

3. Materials and Methods

Four hundred and twenty-two and 411 fecal and clinical samples were taken from five hospitals in Tehran. The fecal flora samples were obtained from patients who were hospitalized more than 3 days and their stools were cultured in azide dextrose broth (Neogen, USA) to isolate

the Enterococcus according to company procedures. The most common clinical samples were urine, blood, wound, ascites, tracheal secretions, stool and urethral discharge of patients.

Resistance to vancomycin was evaluated by disk diffusion method (Mast, United Kingdom) as primary screening test. All Enterococci with vancomycin (30 µg) inhibition zone diameter ≤ 14 mm were collected for minimum inhibitory concentration test and for detection of VLRE; we used agar dilution method in Mueller Hinton (MH) agar medium. Range of vancomycin concentration in MH agar was 2 µg/mL to 1024 µg/mL and *Enterococcus faecalis* ATCC 29212 was used as dilution control of antibiotic. According to CLSI protocols (M100-S17, January 2007), the MIC break point for vancomycin-Enterococcus was 32 µg/mL and values more than 32 and less than 4 µg/mL were considered as VRE and susceptible Enterococcus respectively.

The presence of the vanA and B resistant genes were evaluated by PCR. Amplification of the mixture was performed in a solution consisting of 5 µL of 10x buffer (pH 8.4) containing 100 mM Tris/HCl, 500 mM KCl, 20 mM MgCl₂, 220 µM each dATP, dTTP, dCTP and dGTP, 22 U mL rTaq DNA polymerase, 5 µL bacterial DNA, 5 µL primer and 6 µL H₂O. PCR reactions were performed in an Eppendorf thermo cycler under following conditions: 97 °C for 1 minute (30 cycles), 52 °C for 55 seconds (30 cycles), 72 °C for 1.5 minutes (35 cycles) and final extension at 72 °C for 10 minutes. Amplified products were detected by agarose gel electrophoresis using 1.5% agarose (w/v) in TBE buffer for 40 minutes at 80 V. *E. faecium* ATCC 51559 and *E. faecalis* ATCC 51299 were used for standardizing the PCR amplification of vanA, vanB and *E. faecalis* ATCC 29212 was used as negative control for PCR.

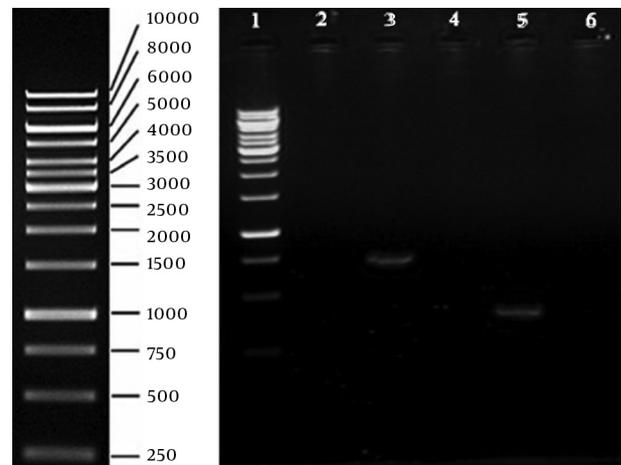
4. Results

A total of 422 and 411 Enterococci were isolated from fecal flora and clinical samples in five general hospitals between March and November of 2012. Two hundred and seventy (65%), 82 (19.5%), 53 (12.5%), 3 (0.8%), 1 (0.3%) of Enterococci were isolated from urine, blood, wound, ascites, endotracheal secretions, stool and urethral discharge samples, respectively. A total of 162 (38.4%) and 159 (38.7%) of Enterococci isolates from clinical and fecal flora samples had MIC's in the range of 16 to 64 µg/ml and were considered as VLRE.

One hundred six (61%) and 125 (39%) of VLREs isolates were *E. faecalis* and *E. faecium*, respectively (Figure 1). Forty three (50%) of VLREs isolates had vanA genotype. Thirty (86%) and 5 (14%) of VLREs with vanB genotype were *E. faecium* and *E. faecalis*, respectively.

Resistance to teicoplanin was detected in 35 (42%) of VLREs with vanA genotype. Sixteen (45%) and 19 (55%) of VLREs with vanB genotype were susceptible and intermediate resistant to teicoplanin.

Figure 1. PCR Amplification of VanA and B Genes



Lane1: Vancomycin Low Level Resistant *E. Faecium* Was Isolated From Urine, Lane 5: Vancomycin Low Level Resistant *E. Faecalis* Was Isolated From Blood. VanA and VanB Positive Isolates Generated a 734 and 420 bp PCR Products, Respectively

5. Discussion

Enterococcus is the second to third common hospital acquired infection and *E. faecium* is an important species in acquired and intrinsic antibiotic resistant (13, 14). Resistance to vancomycin is increased among Enterococci in different countries (15). Although a lot of efforts have been concentrated on studying vancomycin high level resistance Enterococcus, there are very little published studies on VLRE (16-18). VLRE infections are posing a serious problem in healthcare settings because their detection is difficult and treatment of these infections may not be successful because these species are miss-identified as vancomycin susceptible (19-21).

In present study, the presence of vancomycin resistant genes were confirmed by polymerase chain reaction in vancomycin low resistant Enterococcus. Our results showed that 27% and 11% of the VLREs in this study have vanA and vanB, respectively. We also reported that these species can potentially change to vancomycin high resistant and therefore knowledge of vancomycin low level resistant Enterococci rates can contribute to a more precise estimate of vancomycin high resistant Enterococcus rates. Interestingly, there was no difference between rate of vancomycin low level resistant Enterococci isolated from clinical samples and fecal flora samples. In our study, 61% of VLREs were *E. faecalis* which confirms the findings published by other researchers (17, 22, 23).

In this study, all of the VLREs containing vanA were resistant to teicoplanin, 45% of VLREs containing vanB were susceptible to teicoplanin and 55% of isolates showed intermediate resistant to teicoplanin. Teicoplanin resistance patterns in VRE have been reported to depend on presence of vanA or vanB genes (18, 24).

In conclusion, we detected vanA and vanB genes in vancomycin low resistant Enterococci. Our results support that vanA and vanB genes in Enterococci play an essential role in vancomycin resistance. Further studies are required to treat vanA or B positive infections in VLREs in order to devise the best infection control strategies to impede dissemination of those isolates in clinical settings.

Authors' Contributions

Omid Teymourejad: Study concept and design; Dr Ashraf Mohabati Mobarez: Doing of this proposal in laboratory and drafting of the manuscript; Dr Reza Hosseini Doust: Analysis and interpretation of data; Dr Somaye Yaslianifard: Critical revision of the manuscript.

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