Original Article

Evaluation of Van A-B Genes Frequency in Vancomycin Resistant Enterococci spp., Isolated from Clinical Samples of Imam Hossein Teaching Hospital in Tehran

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Abstract

Background: Antibiotic resistance is an important cause of treatment failure and re-infection in enterococci. In this study, the frequency of phenotype and genotype of Van A-B genes in Vancomycin resistant enterococcus isolated from a clinical sample of Imam Hossein Hospitals in Tehran was determined.

Materials and Methods: In this descriptive cross-sectional study in 2018, a total of 76 vancomycin-resistant enterococci in Imam Hossein Hospitals in Tehran were evaluated, including those from blood, urine, sputum, and wound. The frequency of phenotype and genotype of Van A-B genes in them was determined by MIC Epsilometer test and Multiplex Real-time PCR.

Results: The 160 isolates of enterococci collected from different hospital wards revealed that 76 (47%) enterococci were resistant by applying MIC E-test; interestingly, all VRE isolated showed high-level vancomycin resistance. The Real-time-PCR assay demonstrated vanA gene in 76 (100%) VRE isolates. Considering to controls no van B gene was detected in this assay. Based on bacterial phenotype tests, the results showed that 82% and 18% of the isolates were *E. faecium and E*. faecalis, respectively.

Conclusion: Totally, it may be concluded that Van A gene is more accompanied by high-level Vancomycin Teicoplanin resistance in common enterococci species. The frequency of Vancomycin resistance entrocooci is increasing especially among ICU admitted patients. For effective treatment MIC test and Van A-B genotyping seem to be essential.

Keywords: van A, van B, Vancomicin resistance entrococci, Real-time PCR

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Introduction

In the last two decades, enterococci have developed widespread resistance to a number of antibiotics, which has complicated the treatment of infections caused by this bacterium. Enterococci are present in the gastrointestinal tract of humans and animals, in soil, water, and food, and they can grow in environments with high salt concentrations and a wide range of pH. Enterococci have the ability to acquire drug resistance as well as other pathogenic factors. Today, enterococci resistant to various concentrations of Vancomycin are

increasingly being reported from around the world. The van B and van A, genes in enterococci are responsible for resistance to high vancomycin concentrations.

Contributing factors include colonization with resistant enterococci, hospitalization in the intensive care unit, long-term use of antibiotics, and length of hospital stay. Enterococci are mainly transmitted from one patient to another through hospital staff who can carry them themselves. There are 12 species in the genus *Enterococcus*. *Enterococcus faecalis* (*E. faecalis*) is the most common species and is the cause of 85-90% of enterococcal infections and *Enterococcus faecium* is the cause of 5-10% of infections[1]. Usually, 800,000 enterococcal infections occur annually, costing about $ 500 per case[2].

Methods

Demographic information

In this study, patient information including age, sex, history, length and wards of hospitalization, history of antibiotic consumption, the clinical outcome of the patient, underlying disease (immunodeficiency, neutropenia, renal failure and dialysis, cancer and major surgery, severe cardiovascular disease, blood infection, lupus, diabetes), and history of antibiotic use before colonization were extracted from hospital information system (HIS).

Phenotypic experiments

During nine months in 2018, clinical samples, including blood, urine, sputum, and wounds, were collected from Imam Hossein University Hospital, Tehran, Iran.

The patient's samples were obtained after seven days of admission and transferred to the core laboratory on thioglycollate, Stuart's transport medium. All samples were collected from the surgery, internal medicine, pediatric, gynecology, and intensive care unit (ICU) wards. Samples were cultured on sheep blood agar, chocolate agar, McConkey agar and based on the sample type in thioglycollate medium. The sterile body fluids were inoculated to BacT/ALERT bottles.

Bacterial isolates were identified by gram staining, catalase tests, 6.5% saline medium growth, and Bile Esculin hydrolysis test. The ability to use arabinose and sorbitol was used to determine the species of enterococci. For vancomycin resistance screening by disk diffusion method, lawns of each bacterial suspension at a concentration of 0.5 McFarland were made on Mueller Hinton's agar using sterile cotton swabs. Then 30µg vancomycin disks (Rosco®) was positioned on bacterial lawns and incubated at 37°C for 24 hours. The growth inhibition zones greater than 14 mm diameters were considered as vancomycin resistance phenotype (CLSI 2019). Enterococci isolates were checked for Vancomycin, the minimum inhibitory concentration (MIC), using E-test strips (Liofilchem®). All vancomycin-resistant isolates in the disk diffusion method were inoculated on Mueller Hinton's agar with the turbidity of 0.5 Mcfarland, and then the E-test strips were placed on the agar surface and incubated at 37°C for 24 hours. The MIC results of Vancomycin were interpreted according to the E-test strips manufacturer's guidelines and the clinical and laboratory standards institute (CLSI) 2019 instructions.

Molecular experiments

Enterococci colonies of overnight cultures were transferred to the BHI broth medium with 20% glycerol and were stored at -20°C until molecular testing. DNA extraction was performed automatically by magnetic-bead technology, MagCore®, according to the manufacturer's instructions, and the amount of purification was controlled by measuring the OD 260/280 optical absorption ratio. Standard enterococcal strains including *E. faecalis* ATCC1394 and *E. faecalis* ATCC1237 were also positive controls for Van A and Van B genes, respectively (Figure 1).

The multiplex PCR diagnostic test was implemented and optimized on the SLAN® thermocycler according to the kit protocol. Three channels of FAM (Van A), HEX, (Internal control) Cy5 (Van B) were used, and then the obtained amplification curve was examined and analyzed. Positive and negative commercial controls were used in each run, and the amplification curves were interpreted as positive, negative, and unacceptable according to the kit manufacturer's instructions. The Ct value of more than 38 was considered as a negative. The determination of the internal control curve in the appropriate threshold cycle was necessary for negative samples (Figure 2). According to the reaction-making guide (Genproof® VRE PCR), the specificity for these two genes in *E. faecium E. faecalis* and *E. gallinarum* species is close to 100%, and the analytical sensitivity of the method for

Van A and Van B genes was considered 1.398 copy/µl and 1.026 copy/µl, respectively.

Data analysis method

Data analysis was performed using SPSS software version 25.

Ethical considerations

In this study, the information of the subjects remained confidential, and no change was made in the diagnostic and treatment process of the patients, and no cost was imposed on the patients. The Research Deputy approved the mentioned research of Shahid Beheshti University of Medical Sciences with the code of ethics (IR.SBMU.MSP.REC.1397.323).

Results

The mean age of the patients was 61 years with a standard deviation of 22 years, and the sex of patients was 36% male and 64% female. The distribution of patients in hospital wards included 35% ICU, 17% in surgical wards, and 48% in non-surgical wards. The average length of hospital stay was 16 days. The sample was estimated in 4.8% of ascites, 2.4% of pleural fluid, 56.6% of urine, 14.5% of blood, 8.4% of wound or operation site, and 13.3% of other parts.

In 81% of patients, several antibiotics were used. Underlying disease was cancer in 24%, sepsis in 12%, diabetes in 19%, fractures in 6%, and 34% in others. 64% of the patients recovered and were discharged, but 36% of the patients died. Out of 160 enterococci isolated, 76 isolates based on disk diffusion, MIC determination, and molecular studies were resistant to Vancomycin with a frequency of 47%. The MIC value of all resistant isolates was greater than 256 µg / ml (high resistance) (Figure 1). Real-Time PCR results showed that all resistant enterococci were positive for the Van A gene and negative for the Van B gene. Based on diagnostic tests to determine species of resistant enterococci, *E. faecalis* were reported 18% and *E. faecium* 82%.

Discussion

Due to the importance of identifying resistant enterococci in order to take preventive measures and improve the course of antibiotic treatment, in this study, we examined the frequency of Van A and Van B gene phenotype in vancomycin-resistant enterococci isolated from clinical samples of Imam Hossein Hospital during 2018 and it was found that Van A gene was positive in all resistant isolates and Van B gene was negative.

In 2017, Hosseini et al. evaluated the genotype of 48 vancomycin resistance enterococci collected from the urine samples of hospitalized and outpatients in Jahrom. They reported that 28% of isolates were identified as *Enterococcus faecalis*, 12% as *Enterococcus faecium* and 60% were non-faecalis/non-faecium. In our study, 18.1% of resistant enterococci were *E. faecalis* and 81.9% were *E. faecium*, Hosseini et al. also reported that a total of 21 isolates, 75% of the isolates were resistant to Vancomycin in which 4 strains (40%) were positive for van A gene and 2 strains (20%) for vanB gene respectively[3]. However, in our study, 47% of enterococci were vancomycin resistance and the Van A gene was positive in all isolates but none of them carried the Van B gene. These differences in Prevalence of different species of enterococci, resistance profile, and genes in comparison to our study may be due to geographical differences in enterococcal species distribution and vancomycin resistance patterns.

In the study of Vahhabi et al. 432 stool or rectal swab samples were collected from patients who were hospitalized in high-risk wards (ICU, hematology/oncology, infectious and burn) for at least 72 hours, as well as outpatients. They reported 64.9% *E.faecalis* and 29.5% *E.faecium* which was higher and lower than our results respectively. In addition, they also observed the presence of van A or van B genes in 19% of the strains. In contrast to our study, they reported that the frequency of van B was higher than Van A[4]. Our research was not limited to stool samples, and this could be the reason for the difference in results.

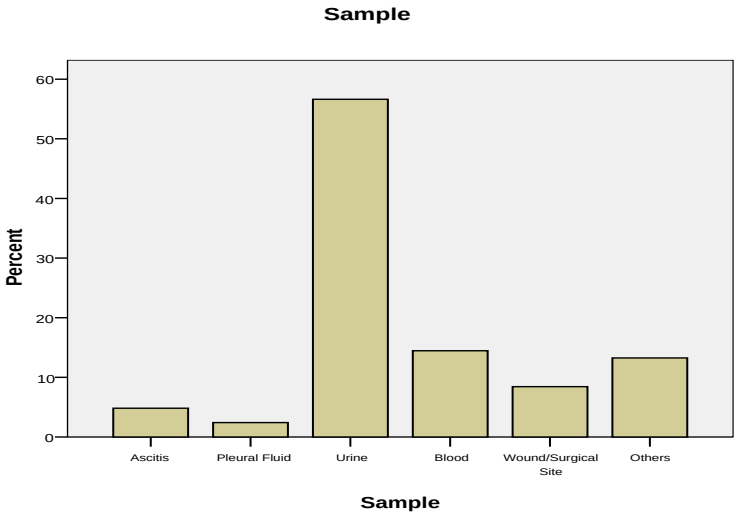

Chart 1. The frequency of vancomycin resistant enterococci isolated from clinical samples.

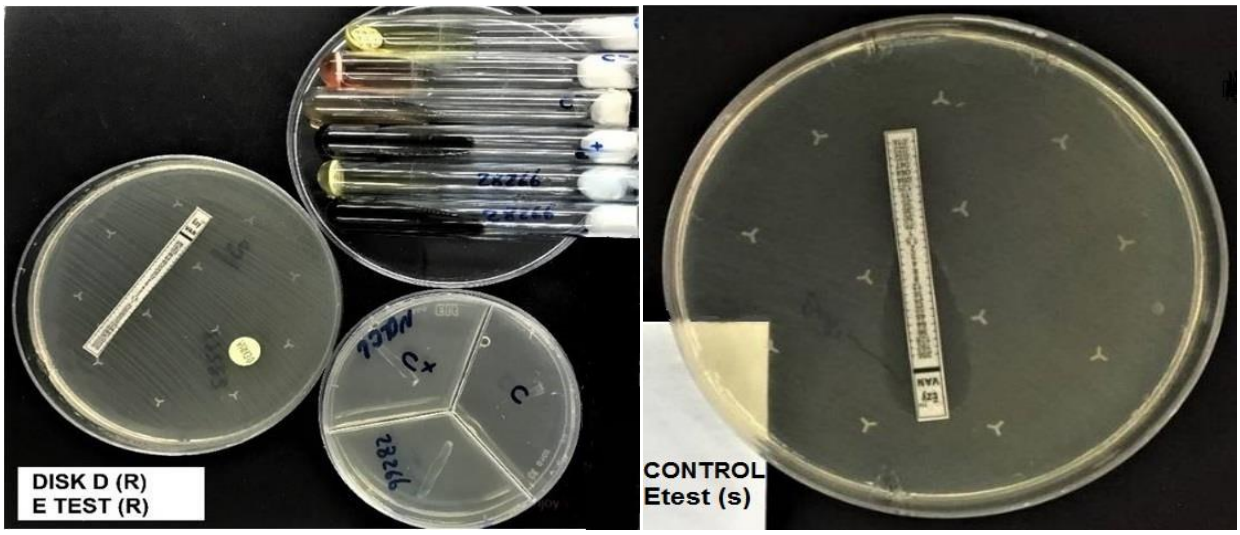

Figure 1. Antibiotic resistance and biochemical tests of enterococci isolates.

In the study conducted with Nikooei et al. in 2018, 165 enterococcal isolates were recovered from clinical specimens in different hospital wards. They reported that out of the 165 isolates, 79 (48%) were resistant to Vancomycin by disk diffusion method, whereas by E-test, only 40 (25%) isolates showed high vancomycin resistance levels. Genotype analyzes by real-time PCR, was exhibited out of 40 samples, 37 isolates (92.5%) contained Van A gene which was analogous with our results and 3 (7.5%) isolates was positive for Van B gene which was in contrast with ours[2]. This finding indicates that one of the important reasons for the different between the results in various studies can be the type of method used for genetic testing.

Masoumi et al. in 2016 investigated that from 278 enterococci isolates 70.86% of the strains were E. faecalis, 15.46% E. faecium, and 13.66% were other Enterococcus species. In comparison, Fasium species showed higher prevalence in this study[5].

Similar to our findings Mohammadi et al. have reported a high incidence of vanA gene (12/15) among 15 vancomycin-resistant enterococci isolates while the vanB gene was not observed in any of the strains[6]. The

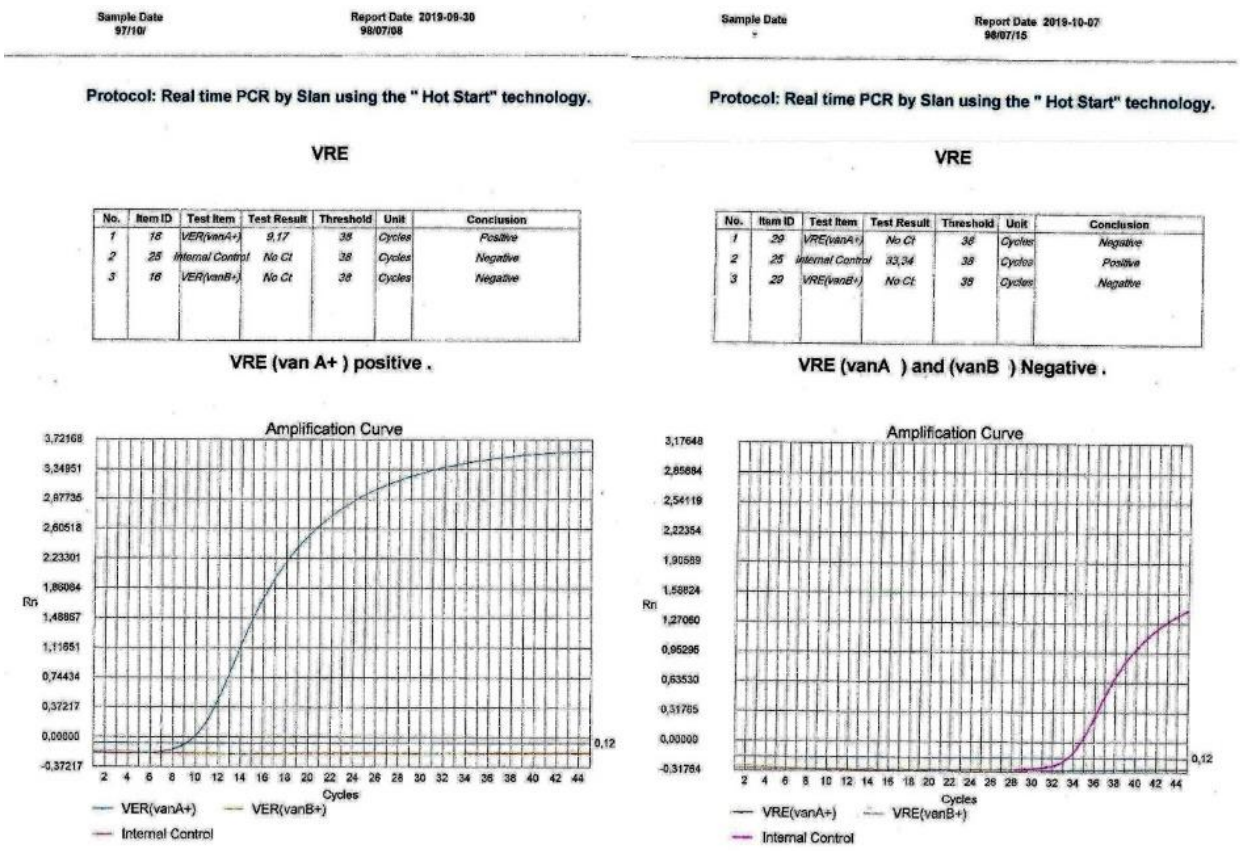

Figure 2. Real time PCR of resistance genes shows Negative and positive vanA controls.

prevalence of gene A was similar to the findings of this study.

Shahraki et al.[7] in 2017 on 113 isolates have reported 92%, 6.2%, and 1.8%, enterococci from urine samples, blood culture, and pulmonary secretions, respectively. This study found that Enterococcus faecalis and Enterococcus faecium were the most resistant to vancomycin and teicoplanin antibiotics, and Van A gene was isolated in all of these samples, and the van B gene was absent in all of them, which was almost consistent with the results of this study.

In another study, Arbabi et al. in 2016 found that out of 160 enterococci collected from Rasoul Akram Teaching Hospital in Tehran, 37% of isolates were vancomycin-resistant, which were phenotypically confirm by broth microdilution method[8].

In a descriptive cross-sectional study of Najafi et al., among 175 enterococci strains, the rate of antibiotic resistance in Enterococcus faecium was higher than that observed in Enterococcus faecalis strains[9]. However, in our study, all *E. faecium* isolates showed a higher prevalence and were Van A positive.

Conclusion

In this study, 160 enterococcal isolates were collected in 9 months. Seventy-six resistant isolates were selected according to the disk diffusion method and MIC confirmation. Then Multiplex Real-time PCR was used for genotype (VanA, VanB) determination. Overall, based on the results of this study, it is inferred that the positiveness of Van A gene more than other genes was associated with antibiotic resistance in different strains of enterococci, and in all strains, there was phenotypic, genotypic coordination. Vancomycin-resistant enterococci (VRE), due to rapid spread, association with infections and high mortality, limited treatment options, and the possibility of transmitting vancomycin-resistant genes to other pathogens and more common pathogens such as *Staphylococcus*

aureus, has become important hospital-acquired pathogens of ESKAPIE Group. Genotype VanA has shown high-level resistance for Vancomycin and teicoplanin. All of these studies have reported high-level resistance (MIC=64 µg / ml) (Figure 1). In this study, the screen test for vancomycin resistance results by broth or Agar dilution method was reported six micrograms per milliliter. It is necessary to use the MIC method to confirm phenotypic tests, so in this study, E- test was used to determine the MIC[10]. According to many meta-analyzes studies, the prevalence of enterococci is increasing in the Middle East, especially in Iran[12, 13]. Asadollahi et al. from the Pasteur Institute of Iran, in a systematic review of articles from 2001 to 2016, have reported a significant prevalence of resistant enterococci in Iranian hospital isolates[11].

The reason for this increase of resistance gene is transmissible plasmid[14]. The responsible genes for vancomycin resistance are called the van gene, which types A and B carry on the Tn1546 transposon. This transposon can enter the conjugative plasmid[15,16]. These two genes (Van A and Van B) cause high resistance to Vancomycin. However, Van C, Van D, and Van E genes cause low-level resistance.

Vancomycin is currently one of the most effective antibiotics in the treatment of enterococcal infections. Enterococci are naturally resistant to cephalosporins but should be routinely tested for penicillin and vancomycin resistance. The acquisition of vancomycin resistance genes occurs in different ways and by various mechanisms, the most common of which is transferring van A-B genes through the transposons[17].

For clinicians, identifying the Van A gene means high-level resistance to Vancomycin and teicoplanin. The antibiotic of choice in these cases is linezolid or operzolide. Since this resistance is increasing among bacterial strains, rational antibiotics prescription is essential. In addition, identification of Van A and Van B genotypes may be helpful in the treatment of such resistant infections.

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