

GeneXpert *vanA/vanB* test and culture method in evaluation of Vancomycin Resistant Enterococcus (VRE) colonisation in a tertiary hospital

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Abstract

Background: Enterococci are responsible for various nosocomial infections. The spread of vancomycin-resistant enterococci (VRE) especially in intensive care units (ICU) is a major threat in hospitals. Determination of VRE colonisers is crucial in order to prevent spreading of VRE. It was aimed to determine the colonisation of VRE among ICU patients by GeneXpert *vanA/vanB* test and culture method in a period of two years.

Method: From April 2015 to March 2017, a total of 788 rectal swab specimens were obtained from 292 ICU patients hospitalized at Dicle University hospitals. Swab samples were evaluated for *VanA* and *VanB* genes by GeneXpert® *vanA / vanB*, (Cepheid, USA) real time polymerase chain reaction (Rt-PCR) commercial system. Enterococcosel agar with 8 µg/mL vancomycin was used for cultivation of the samples. Swab samples were inoculated at 37°C, in aerobic condition for 48 hours. The growing bacteria were identified by Maldi Biotyper (Bruker, Germany) and antimicrobial susceptibilities were carried by BD Phoenix (Becton Dickinson, U.S.A) automated microbiology system.

Results: Among 788 swab samples 116 (14,7%) were detected as VRE with GeneXpert *vanA/vanB* method. Of PCR positive samples, 107 (92,2%) were *VanA*, 7 (6%) were *VanB* and 2 (1,7%) were both *VanA* and *VanB* harbouring. Among *VanA* detected samples, 75 grew *Enterococcus faecium*, 8 grew *Enterococcus faecalis* isolates while 24 samples had no growth in cultivation. Among 7 *VanB* harbouring samples, 1 was identified as *E. faecium* while other 6 had no growth. Two samples with both *VanA* and *VanB* genes were isolated as *E. faecium*.

Conclusion: Active VRE surveillance and isolation of VRE carriers is crucial in hospitals. Molecular methods have important advantages in early diagnosis and on time isolation of the patients.

Keywords: vancomycin-resistant enterococci, *vanA / vanB*, intensive care unit

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Introduction

Enterococci are responsible for various nosocomial infections. They possess intrinsic and acquired antimicrobial resistant mechanisms. Resistance to glycopeptides due to the acquisition of *vanA* and *vanB* genes is a major threat in healthcare settings^{1,2}. Vancomycin resistant Enterococci (VRE) infections lead increased morbidity, mortality and hospital costs. Early and accurate detection of VRE is important not only for adequate treatment, but also for preventing the transmission of resistant pathogens³.

Culture is the “gold standart” but time consuming method for VRE detection. It takes 48 to 72 hours to accurately identify a VRE isolate. Molecular based automated in vitro test assays for the rapid detection of VRE directly from swab samples came into use in the last decades^{4,5}. The GeneXpert system (Cepheid, USA) is an automated real time polymerase chain reaction (PCR) system providing results approximately in one hour’s time. Nucleic acid sample preparation, amplification of enterococcus DNA and real-time detection of *VanA* and *VanB* genes are performed in a macro/microfluidic cartridge with the GeneXpert Dx system instrument⁶.

It was aimed to evaluate VRE colonisation among ICU patients of a tertiary hospital by cultivation and GeneXpert system.

Materials and Methods

A total of 788 rectal swab specimens were obtained from 292 patients hospitalized in ICUs at Dicle University hospitals. Patients newly admitted to ICUs from April 2015 to March 2017 were enrolled in the study. Rectal swab samples were collected and transported to the laboratory by Stuart transport medium (HiMedia, India). The swab samples were analyzed for real time PCR by GeneXpert *vanA/vanB*, (Cepheid, CA, USA) automated multiplex real-time PCR assay. The swab sample was added to the elution buffer and vortexed for 30 seconds. The buffer solution was transferred to a single-use cartridge with reagents and integrated chambers. The preparation of the cartridge took approximately five minutes. The prepared cartridge was placed in the device. the Gene Xpert™ Dx module and *VanA/VanB* assay was selected and executed. In about one hour’s time the results were viewed as *VanA* or *VanB* positive or negative.

Swab samples were cultivated in enterococcosel agar (Oxoid, England) with 8µg/ml vancomycin and were incubated at 37°C in aerobic conditions. The bacterial growth was controlled at 24, 48 and 72nd hours. Black colonies with esculin-positivity were subcultured on 5% sheep blood agar (Oxoid, England). The growing bacteria were analysed with MALDI-TOF MS (Matrix assisted laser desorption mass spectrometry) method. The bacterial colony was applied to the stainless steel plate with the help of a toothpick. Formic acid solution and HCCA matrix solutions were applied after drying of the steel plate. Spectrum images of the bacteria exposed to laser shots in the Microflex device were compared with database images and finally the bacteria were identified up to species level by Maldi Biotyper (Bruker, Germany). Antimicrobial susceptibility testing was carried out with BD Phoenix (Becton Dickinson, U.S.A) automated system according to the European Committee on Antimicrobial Susceptibility Testing breakpoint tables⁷. The isolates detected as vancomycin resistant were also tested by Kirby Bauer disc diffusion method. .

Statistical analysis

Categorical variables were displayed as frequencies (%), Pearson's Chi Square Test was performed for comparing tests.

Results

Among 788 rectal swab samples 116 (14,7%) were detected as VRE with GeneXpert® vanA / vanB, (Cepheid, USA) rt-PCR system. Of 116 PCR positive samples 86 yielded VRE by culture whereas 30 remained negative. A total of 91 samples yielded VRE by culture; 86 of them were detected as VanA and/or VanB harbouring while 5 samples harboured none of the genes by GeneXpert/VanA-VanB test. Comparison of culture and GeneXpert/VanA-VanB assay was summarized in Table 1. Performance of the GeneXpert/VanA-VanB assay was determined using the culture as the gold standart method. The sensitivity and specificity of GeneXpert/VanA-VanB assay were 94,50% and 95,69% respectively. The negative predictive value (NPV) of the test was high (99,25%) while positive predictive value (PPV) was relatively low (74,13%).

Table 1. Comparison of culture and GeneXpert/VanA-VanB test

| GeneXpert/VanA-VanB assay | Culture | | Total n |
|---------------------------|------------|------------|---------|
| | Positive n | Negative n | |
| Positive n | 86 | 30 | 116 |
| Negative n | 5 | 667 | 672 |
| Total n | 91 | 697 | 788 |

Of PCR positive samples, 107 (92,2%) were detected as VanA, 7 (6%) as VanB and 2 (1,7%) as both VanA and VanB harbouring. Among VanA detected 107 samples, 75 grew *Enterococcus faecium*, 8 grew *Enterococcus faecalis* while 24 samples had no growth in cultivation. Among 7 VanB harbouring samples, 1 was identified as *E. faecium* while other 6 samples had no growth. *E. faecium* was isolated in 2 samples harbouring both VanA and VanB genes. None of VanA or VanB genes were detected in 5 rectal swab samples of which culture grew *E. faecium* (Table 2).

Table 2. GeneXpert/VanA-VanB test positive samples

| | | Culture results | | | |
|--|------------|-----------------|-------------------|--------------------|-------|
| | | No growth | <i>E. faecium</i> | <i>E. faecalis</i> | Total |
| Detected gene(s) by GeneXpert/VanA-VanB test | VanA | 24 | 75 | 8 | 107 |
| | VanB | 6 | 1 | - | 7 |
| | VanA+ VanB | - | 2 | - | 2 |
| | None | 667 | 5 | - | 672 |
| | Total | 697 | 83 | 8 | 788 |

Discussion

Glycopeptide-resistant enterococci (GRE) was first described by Uttley et al. in Europe in 1988⁸. Glycopeptide resistance is based upon producing modified peptidoglycan precursors which show decreased affinity for glycopeptide antibiotics such as vancomycin and teicoplanin. VanA was the most reported acquired resistance gene, while reports about VanB were relatively rare. VanC stays as an intrinsic resistance gene in some *Enterococcus* species such as *E. casseliflavus* and *E. gallinarum*. VanD, VanE and VanG are the other reported genes for glycopeptide resistance in *Enterococci*⁹. The transmission of VanA and VanB genes among bacteria poses a serious threat to healthcare settings Active infection-control interventions including surveillance cultures and the isolation of colonised or infected patients were recommended to prevent the transmission of VRE in the health care settings^{10,11}. Our study was a part of the infection-control intervention of our hospital reduce the spreading of VRE, especially in ICUs.

Microbiology laboratories are the first steps in surveillance studies of VRE. The early and accurate detection of patients colonised with VRE is crucial for implementation of the contact isolation precautions. The culture method remains as the gold standard for detection of VRE but incubation and identification processes takes 3-5 days. Conventional molecular methods have been used for VRE detection, but they were not useful for routine laboratory use¹¹. The Gene Xpert vanA/vanB assay was described as an easy to use, rapid and accurate real time PCR method for detecting VanA and VanB genes from perianal/rectal swab samples of colonized patients. The assay performs DNA extraction, PCR amplification, and detection steps in a closed automated system. All steps of the real-time PCR process takes less than one hour with this rapid assay^{4,6}. In our study, both culture and Xpert vanA/vanB assay were used to detect VRE colonisation of ICU patients. The sensitivity and specificity of GeneXpert/VanA-VanB assay were found as 94,50% and 95,69% respectively. in our study. A high value (99,25%) of NPV indicates that the sample with a negative result of Xpert assay is indeed not VRE with a probability of 99,25%. Such a result will lead to eliminating the cultural processes of the sample. Bourdon et al., reported the sensitivity and NPV of Xpert test as 100%, compared to enriched culture in a study of 804 swab samples⁶. In a study during outbreaks of vanA-positive *E. faecium* in university hospitals of Copenhagen, Holzkecht et al. analysed 1110 samples and reported a negative predictive value of 97,7% for Xpert assay¹². An NPV of 97,4% was reported by Uludağ Altun et al., in a study including 210 perirectal swab samples from Turkey¹³. Zhou et al. reported an NPV of 99,5% in a study which rectal swabs and enriched broths were analyzed¹⁴. Babady et al. reported both the sensitivity and negative predictive value of Xpert assay results of 300 rectal swabs as 100%, while the specificity and PPV were reported as 96.9% and 91.3%, respectively¹⁵. Negative predictive value of Xpert assay in our study was consistent with the previous studies.

Positive predictive value of Xpert vanA/vanB assay was found as 74,13% in our study. It indicates that a sample with positive result of the assay is indeed positive with a probability of 74,13%. Of 107 VanA harbouring samples, 83 grew vancomycin resistant isolates (75 *E. faecium*, 8 *E. faecalis*) while 24 grew none of vancomycin resistant isolates. Of 6 VanB harbouring samples, only 1 grew vancomycin resistant *E. faecium* while any VRE didn't grow in other five isolates. Bourdon et al. also reported a lack of specificity (85.4% specific with a PPV 8.7%) especially about the VanB gene. One of the explanations of the false-positive results was the presence of glycopeptide-resistance genes in uncultured bacteria such as Gram-positive anaerobe bacteria of the gut. The lack of specificity of primer or the probes of Xpert assay and the lack of sensitivity of culture method were the other explanations of the false-positive results of Xpert assay⁶. Holzkecht et al. reported the sensitivity, specificity, positive and negative predictive values of vanA part of GeneXpert assay as 87.1%, 99.7%, 98.0% and 97.7%, respectively. The specificity and positive predictive value of the vanB part of Xpert assay were reported as 77.6% and 0.4%, respectively. The high diagnostic accuracy of vanA part of the assay gave a considerable benefit during VRE outbreaks in Denmark while false positive or negative results were reported to leading to additional transmission risk and isolation days according to the study¹². Zhou et al. evaluated the Xpert vanA/vanB assay on enriched inoculated broths to limit the false-positive results of VanB part by changing the cycle threshold (CT) cut off values of the assay. They recommended confirming the

results with CT values of 25-30 while accepting the results with CT values of ≥ 30 as true negative without further confirmation¹⁴.

Conclusion

GeneXpert VanA/VanB assay provides early detection of VanA and VanB genes in rectal/perirectal swab samples of VRE colonised patients within approximately one hour. The high NPV of the assay leads to reducing the need to culture processes. Whereas the low PPV of the assay, especially due to VanB gene still needs confirmation by culture method. Routine surveillance methods for VRE should be considered according to the conditions of laboratory and health-care settings.

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