Comparison of the BD GeneOhm VanR assay and a chromogenic agar-based culture method in screening for vancomycin-resistant enterococci in rectal specimens of pediatric hematology-oncology patients

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VRE species are an increasingly important and universal problem in intensive care units and hematology-oncology departments due to the spread of glycopeptide resistance. Rapid and accurate identification of VRE is therefore crucial. The intent of this study was to compare the diagnostic performance of a real-time PCR test, the BD GeneOhm VanR assay (GeneXpert vanA/vanB, Cepheid, USA), with conventional cultures for screening hospitalized immunocompromised hematology-oncology patients for VRE. Three hundred and six duplicate rectal swab specimens were obtained from 120 pediatric hematology-oncology patients. PCR and conventional culture-based studies were performed. One hundred and twenty patients, 46 female and 74 male, participated in the study. The mean age of the patients was 7.5±4.7 years. A total of 51 specimens from 306 samples were found to be positive for vanA or vanB. Mean turnaround time for PCR was 0.5±0.2 days. Compared to the culture method, the RT-PCR assay had an overall sensitivity of 91.8% (34/37) and a specificity of 93.6%. The positive predictive value and negative predictive value were 66.6% and 98.8%, respectively. This study demonstrates that RT-PCR is a suitable alternative to culture-based procedures for rapid and accurate identification of VRE in hematology-oncology patients, as the overall performance of PCR is comparable to that of a chromogenic agar-based culture method for VRE screening, especially for detection of VRE-negative patients.

***Key words:** BD GeneOhm VanR assay, chromogenic agar-based culture, vancomycin-resistant enterococci, hematology-oncology.*

Infection with vancomycin-resistant enterococci (VRE) is an important problem in hospitals worldwide[1]. VRE has become increasingly responsible for serious clinical and nosocomial infections[2-5]. The presence of VRE species is a growing and universal issue in intensive care units and hematology-oncology departments in particular, because of the spread of glycopeptide resistance, mediated mostly by the *vanA* and *vanB* genes in enterococci.

The increase in the incidence of enterococcal infections is a result of many factors, including increasing numbers of immunocompromised patients, long and intensive treatment periods in hematology-oncology patients, extensive use of broad-spectrum antibiotics and the spread of multiresistant enterococci. Rapid and accurate identification of VRE is therefore crucial in the management and treatment of both colonized and infected patients and in the implementation

of appropriate infection control procedures to prevent the nosocomial spread of VRE[6]. Culture of rectal swabs has traditionally been used to identify VRE-colonized individuals, but culture-based screening methods for VRE are typically time-consuming and can take from 1 to 5 days to be completed[6-9]. Molecular methods may contribute to reducing the time necessary to obtain results, providing the possibility of an intervention in a more suitable timeframe[10].

The intent of the present study was to compare the diagnostic performance of a real-time PCR test, the BD GeneOhm VanR assay (BD GeneXpert Van A/Van B, Cepheid, USA), with a conventional culture method for screening hospitalized immunocompromised hematology-oncology patients for VRE.

Material and Methods

Three hundred and six duplicate rectal swab specimens were obtained from 120 pediatric hematology-oncology patients between June 2011 and December 2012. Rectal swabs were transported in Stuart transport medium. The allocation of which swab in a set was tested by culture or PCR was random.

Rectal swabs were directly inoculated onto a chromogenic agar plate (chromID VRE agar, bioMérieux, France) containing 8 mg vancomycin ml^{-1} and incubated aerobically at 36°C for 72 hours. Identification and antibiotic susceptibility tests were performed using the automated VITEK-2 system (bioMérieux, France) with gram-positive identification card AST-P592, a supplementary Etest (bioMérieux, Durham, NC, USA) and a disk diffusion test according to the manufacturer's instructions. vanA and vanB resistance phenotypes were reported by the system on the basis of MIC values.

The BD GeneOhm™ VanR assay (GeneXpert vanA/vanB, Cepheid, USA) is a qualitative in vitro test for the rapid detection of vancomycin resistance (*vanA* and *vanB*) genes directly from perianal or rectal swabs. This assay also includes an internal control to detect PCR inhibitory specimens and to confirm the integrity of assay reagents. The assay was performed on an automated real-time PCR instrument. Perianal or rectal swabs were collected and transported to the laboratory using a recommended transport device according to the manufacturer's instructions. The swabs were eluted in sample buffer and the specimens were lysed. An aliquot of the lysate was added to PCR reagents containing the vanA- and vanB-specific primers. Amplified targets were detected with hybridization probes labeled with quenched fluorophores (molecular beacons). The amplification, detection and interpretation of the signals were done automatically by the Cepheid SmartCycler® instrument software.

In this study, the chromogenic agar-based culture method was considered the reference method for VRE screening. The sensitivity, specificity, positive predictive value and negative predictive value of RT-PCR were evaluated in comparison with the results of chromogenic agar-based culture.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software. We looked at sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) to evaluate the accuracy of the RT-PCR method in identifying VRE-colonized patients.

This study was approved by the Research Ethics Board of Dr. Behçet Uz Children's Hospital.

Results

One hundred and twenty patients hospitalized in the pediatric hematology-oncology department between June 2011 and December 2012 participated in this study; 46 (38.3%) of the patients were female and 74 (61.7%) were male. The mean age of the patients was 7.5±4.7 (range: 6 months–18 years) years. The primary diagnoses of the patients included in the study are shown in Table I. Common causes for hospitalization were chemotherapy (48.4%) and febrile neutropenia (21.2%).

Thirteen patients (10.8%) had diarrhea and 87 patients (72.5%) had fever during the period in which the samples were taken. Twenty-four patients (20%) had VRE colonization. A total of 37 VRE were isolated from 306 samples, for an overall culture positivity rate of 12.1% (37/306). All of the VRE recovered were resistant to vancomycin (MIC ≥ 32 μg/ml^{1}) as determined by the VITEK-2 system. Vancomycin MICs were also determined by Etest. All isolates were *Enterococcus faecium*.

A presumptive VRE-positive report was

Table I. Primary Diagnosis of the Patients

Diagnosis	n	%
Acute lymphoblastic leukemia	43	35.8
Neuroblastoma	10	8.3
Acute myeloid leukemia	9	7.5
Wilms' tumor	5	4.2
Rhabdomyosarcoma	5	4.2
Medulloblastoma	3	2.5
Hemophagocytic lymphohistiocytosis	3	2.5
Osteosarcoma	3	2.5
Ewing sarcoma	2	1.7
Germ cell tumor	2	1.7
Hodgkin lymphoma	2	1.7
Other*	33	27.5
Total	120	100

*Other diagnoses: aplastic anemia, ganglioneuroma, hepatoblastoma, pineablastoma, colon cancer, Langerhans cell histiyocytosis, malign glioma, meningioma, myelofibrosis, nasopharyngeal cancer, non-Hodgkin lymphoma, posterior fossa tumor, retinoblastoma

provided the day after submission for the gram-positive cocci that grew on chromID VRE agar, but the median time required from specimen submission to final report was 2 days (range: 1 to 4 days).

A comparison of the results of RT-PCR and chromogenic agar-based culture is shown in Table II. A total of 51 specimens from 306 samples were found to be positive for *vanA* or *vanB* (46 *vanA* positive and 5 *vanB* positive). Mean turnaround time for PCR was 0.5±0.2 days. Compared to the culture method, the RT-PCR assay had an overall sensitivity of 91.8% (34/37) and a specificity of 93.6%. The PPV and NPV were 66.6% and 98.8%, respectively. PCR inhibition was not observed in our study.

Twenty specimens showed discrepant results between culture and PCR: 3 tested culture-positive and PCR-negative; 17 tested culture-negative and either *vanA* or *vanB* PCR-positive.

We had 138 blood samples (45.1%) taken simultaneously with the rectal samples. Eighteen of 138 (13.0%) blood cultures were positive. The most common agent was coagulase-negative *Staphylococcus* (50%), followed by contamination (11%), *Pseudomonas aeruginosa* (6%) and *Escherichia coli* (6%). VRE was not isolated from any of the blood cultures.

Discussion

VRE infection is an important issue for nosocomial infection control. The impact of active surveillance on reduction of the VRE infection rate was demonstrated in studies by Price and Lee[11]. Strategies to control the dissemination of VRE include the detection of VRE carriers. Thus, improvements in VRE diagnostics may improve the rate of early detection of VRE carriers and reduce the risk of VRE transmission. Culture-based procedures are the most commonly used techniques; however, these methods are time-consuming and probably have minimal impact when making decisions for cohorting patients on admission to a hospital ward[1]. The time factor is especially important in oncology-hematology wards, in which patient turnover rates are high due to recurrent chemotherapy protocols, febrile neutropenia episodes and new diagnoses. Insofar as molecular methods reduce turnaround time; they have been reported to have proven their usefulness in the rapid detection of carriers[12,13]. Rapid detection of *vanA* and *vanB* genes is accomplished directly from clinical specimens and enrichment broths by real-time multiplex

Table II. Comparison of Chromogenic Agar-Based Culture and PCR for the *vanA* and *vanB* Genes

		Chromogenic agar-based culture		Total
		Positive	Negative	
PCR	*vanA* positive	34	12	46
	vanB positive	0	5	5
	Negative	3	252	255
Total		37	269	306

PCR assay. However, no consensus exists regarding the most effective approach[14,15]. As turnaround time is one of the most noticeable aspects of laboratory service, use of PCR-based systems for detection of VRE has increased significantly in microbiology laboratories[16]. PCR has advantages over phenotypic methods in that it allows for more timely implementation of infection control interventions by means of reducing the time required for detection of resistance. Studies that have employed PCR for the detection of VRE have reported various results, with different sensitivities and specificities[6,8,9,17]. In this report, we assessed the performance of real-time PCR technology without using an enrichment broth step, a technique that was less labor intensive than conventional PCR methods. PCR methods provide a rapid, sensitive and specific means for the detection and identification of infectious agents while reducing the risk of contamination by a previously amplified product. The results of our study demonstrated that the performance of RT-PCR is comparable to that of culture for VRE screening using rectal swab specimens. The sensitivity and specificity of RT-PCR in our study (91.8% and 93.6%, respectively) were higher than that reported in many other studies on this issue. Previous studies that evaluated the performance of PCR for VRE screening have shown good results for detection of the *vanA* gene. However, low specificity and poor PPV for *vanB*-positive results have been a limitation of the PCR method[15,18]. The presence of the *vanB*-containing transposons Tn5382 and Tn1549 in nonenterococcal anaerobic bacteria, such as *Clostridium* spp., *Eggerthella lenta* and *Ruminococcus* spp., may be a possible explanation for the high *vanB* false positive rate[19,20]. However, only five specimens were positive for *vanB* PCR in our study, and this had little effect on overall performance. *vanB*-containing *Enterococcus* spp. with low-level resistance to vancomycin may have been inhibited by vancomycin at the 8 mg/ml[1] concentration existing in chromID VRE agar, as mentioned in the Çetinkaya and Grabsch reports[21,22]. In this study, no VRE were isolated from *vanB* PCR-positive specimens.

In our study, the majority of the results that were discordant between PCR and culture were observed in *vanA*-positive specimens. There are three reasons that may explain this situation. Firstly, a limited number of microorganisms, such as *Bacillus circulans*, *Arcanobacterium haemolyticum*, *Oerskovia turbata* and *S. aureus*, have been reported to have acquired *vanA* genes[23-25]. So, such nonenterococcal *vanA*-harboring isolates may contribute to false-positive results. Secondly, the presence of a rectal swab specimen containing a low concentration of VRE could also be a factor influencing the observed sensitivity of the culture method, yielding a false-negative result in a case where a resistance gene would be detected by the PCR method[26]. Thirdly, nonviable or viable but nonculturable *Enterococcus* spp. may yield culture-negative but PCR-positive results[27]. In some previous studies, this problem—culture-negative and PCR-positive results—was resolved by a broth enrichment step prior to culture. However, the third explanation would be least likely in our study, since we did not use a broth enrichment step for culture[28,29]. We had three PCR-negative but culture-positive results. According to a previous report, mutations within the primer-binding sequence may yield false-negative results[30]. This would be an explanation for our PCR-negative but culture-positive results. In our study, we likely found low PPV due to the reasons explaining false positive PCR results mentioned above. But our NPV was as high as that seen in previous reports, which is more important for the rapid, accurate identification of VRE—the latter being crucial

in the management and treatment of colonized and infected patients and the implementation of appropriate infection control procedures to prevent the spread of VRE.

In this study, we focused on the performance of RT-PCR in a specific group: patients hospitalized in the oncology-hematology department. Hospitalization for long periods, neutropenia and use of antibiotics in patients with malignancies have been found to be associated with VRE colonization/infection. Since VRE is an important nosocomial pathogen, strict infection control measures should be implemented. These include cohorting patients and nurses on the basis of VRE colonization, which entails segregating and setting aside patient rooms. This is difficult to do in departments like ours, with high patient censuses and high rates of patient turnover. The results of this study, with high negative predictive values for RT-PCR, could help in developing a new strategy for admitting patients to the service and cohorting them. This would reduce VRE transmission in an oncology unit where VRE was endemic.

In conclusion, this study demonstrates that RT-PCR is a suitable alternative to culture-based procedures for the rapid and accurate identification of VRE in hematology-oncology patients, as the overall performance of PCR is comparable to that of a chromogenic agar-based culture method for VRE screening, especially for detection of VRE-negative patients.

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