Rapid detection of Van genes in rectal swabs by real time PCR in Southern Brazil

Vladimir Cantarelli 1, Bianca Cavalcante 1, Diogo André Pilger 1, Fabiana Souza 1, Cicero Gomes Dias 2, Teresa Brodt 1, Maritza Cantarelli 3, Carina Secchi 1 and Pedro Alves d’Azevedo 2

ABSTRACT

Introduction: Laboratory-based surveillance is an important component in the control of vancomycin resistant enterococci (VRE). Methods: The study aimed to evaluate real-time polymerase chain reaction (RT-PCR) (genes vanA-vanB) for VRE detection on 115 swabs from patients included in a surveillance program. Results: Sensitivity of RT-PCR was similar to primary culture (75% and 79.5%, respectively) when compared to broth enriched culture, whereas specificity was 83.1%. Conclusions: RT-PCR provides same day results, however it showed low sensitivity for VRE detection.

Keywords: RT-PCR, Vancomycin resistant enterococci. Genes vanA-vanB.

RESUMO

Introdução: Vigilância com base em detecção laboratorial é um componente importante no controle de enterococos resistentes a vancomicina (ERV). Métodos: Avaliamos procedimento da reação em cadeia da polymerase real time (PCR-RT) (genes vanA-vanB) para detecção de ERV em 115 swabs de pacientes incluídos em um programa de vigilância. Resultados: A sensibilidade do RT-PCR foi semelhante a da cultura primária (75% e 79,5%, respectivamente) quando comparada com a cultura em caldo enriquecido, enquanto a especificidade foi de 83,1%. Conclusões: O RT-PCR fornece resultados no mesmo dia, contudo mostra baixa sensibilidade para a detecção de VRE.

Palavras-chaves: RT-PCR, Enterococos resistentes à vancomicina. Genes vanA-vanB.

Real time polymerase chain reaction (RT-PCR) is being used with two pairs of primers designed to amplify the vanA and vanB genes using SYBR Green-based reagents on the Light Cycler platform (Roche). Different studies used diverse approaches (use of enrichment broth was not performed in all studies, for instance), which makes inter-study comparisons inconsistent. Overall, the performance of the PCR-based procedures showed promise, since screening can rule out negative patients in up to 24h.

The present study intended to compare an in-house rapid cycle real-time PCR assay with culture methods for VRE using rectal swabs from hospitalized patients in an endemic setting.

Rectal swabs from hospitalized patients at Hospital Ernesto Dorneles, Porto Alegre, RS, Brazil, collected from September 2006 to April 2008 were used. The institution has 300 beds, 22 of which are intensive care unit beds, and VRE are endemic in this hospital. VRE screening (rectal swabs) is performed once a week for all new patients admitted to the ICU and those showing positive results.

Samples were inoculated onto EnterococcusSel™ agar (BBL, USA) supplemented with 6 µg of vancomycin per mL and incubated at 35°C for up to 72h. Suspicious colonies (Esculin positive) were planted onto sheep blood agar (BioMérieux, Brazil) and both resistance to vancomycin and identification were confirmed using disk diffusion and/or the Vitek system, following Clinical Laboratory Standards Institute criteria for definition of resistance.

Following the conventional procedure for VRE screening, rectal swabs were inoculated into Brain Heart Infusion (BHI, Difco, USA) broth, supplemented with gentamicin (8 µg/mL) plus vancomycin (6 µg/mL). Inoculated broths were incubated for 18h and then an aliquot was used to inoculate a new enterococcosel agar plate, as above. Culture after broth enrichment was used as the reference method.

After a 6h incubation in BHI-GV broth, 200µL were collected and used for DNA extraction with the QIAamp DNA Mini Kit (Qiagen), in accordance with the manufacturer’s instructions. Two µL of the extracted DNA were used in a total volume of 20mL of PCR mix containing SYBR Green II (Invitrogen). A new set of primers were designed to amplify both the VanA (169bp) and VanB (101bp) genes: VAN-A1-LC: 5’-AGCTGTACTCTCGCGGATA-3’ and VAN-A2-LC: 5’-CGCAGGCTACAGAAGGATA-3’; VAN-B1-LC: 5’-AGGCGGAGGAGCTTACCTAC-3’ and VAN-B2-LC: 5’-AGGCCGAGACGCTTACCTAC-3’. Real time PCR were performed using the LightCycler platform (Roche) and distinction between VanA and VanB alleles was achieved by melting curve analysis (VanA: 79°C, VanB: 83°C).
A total of 115 rectal swabs were obtained. VRE Screening using broth enrichment detected 44/115 (38.6%) positive cultures, 28/115 (24.3%) were Enterococcus faecalis (n=28/115), 6/115 (5.3%) were E. gallinarum (n = 6), and 1/115 (0.9%) were E. casseliflavus. Positive results were observed in 35/115 (30.4%) and 45/115 (39.1%), respectively, when VRE screening (primary planting) and molecular screening for VRE were used. Among the 45 positive PCR results, in only one sample vanB gene was detected. False positive results for PCR were observed in 12 (11 vanA and 1 vanB) samples, whereas no false positive results were observed when primary plating was used. False negative results were verified in 9/115 (7.8%) and 11/115 (9.6%) of the samples tested for primary planting and PCR, respectively. Among the six isolates of vancomycin-resistant E. gallinarum, molecular screening was positive in three samples, whereas in the sample containing E. casseliflavus, a positive PCR result was obtained. A summary of results for VRE screening is shown in Table 1.

Strategies to control the dissemination of VRE include the detection of carriers and culture based procedures are used for this purpose1. These methods are time consuming and vary in performance according to the procedure used. Molecular methods reduce turnaround times; however no consensus exists regarding the performance according to the procedure used. Molecular methods may be necessary to definitively resolve this issue.

In the present study, both primary culture and molecular screening showed low sensitivity (79.5% and 75%, respectively) in the detection of VRE carriers. False negative results of the molecular screening may be due to the presence of inhibitors in the samples and the introduction of an internal control and/or a better extraction procedure may be necessary to achieve improvement. Moreover, the six hour incubation of the broth may be not sufficient as a pre-amplification period. The molecular screening also showed low specificity (83.1%) and some possibilities may be considered at this point. False positive results (molecular screening positive and culture negative) were also observed by other authors and may be due to nonviable or nonculturable enterococci. On the other hand, inhibition to 6µg/ml of vancomycin may explain the false-positive result in the vanB gene containing sample.

One interesting aspect was the presence of vancomycin-resistant species other than E. faecalis or E. faecium in a relatively high number of samples (E. gallinarum = 6, E. casseliflavus = 1). The presence of E. gallinarum containing vanA isolates has already been described in Brazil and deserves special attention in future studies10. Discrepant results for the molecular screening were observed in half of these samples.

The control of VRE dissemination is a complex process in which the laboratory plays an important role. According to results of the present study, due to its low sensitivity, molecular screening cannot replace a highly sensitive method (broth enrichment culture), in the detection of VRE carriers. This is also true for primary planting culture with selective medium. The potential benefits of a 24h turnaround time and the consequent earlier intervention must be researched, since the reference method used in this study is laborious and time consuming for VRE detection. Clinical studies, with patients managed following detection of VRE by a molecular method or culture and evaluation of outcomes, including infection and mortality due to VRE, may also be necessary to definitively resolve this issue.

| TABLE 1 - Sensitivities, specificities and predictive values for primary planting and molecular screening for VRE compared to VRE screening using broth enrichment for detection of VRE. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Sensitivity     | Specificity     | Positive         | Negative         |
|                  | n    | %    | n    | %    | predictive value | n    | %    | predictive value |
| Primary planting | 35/44 | 79.5 | 71/71 | 100.0 | 35/35 | 100.0 | 71/80 | 88.8 |
| Molecular screening (PCR) | 33/44 | 75.0 | 59/71 | 83.1 | 33/45 | 73.3 | 59/70 | 84.3 |

VRE: vancomycin resistant enterococci, PCR: polymerase chain reaction.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FINANCIAL SUPPORT

This work received funding from the ANVISA/OPAS - BR/LOA/0800015.0001 and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES