Communication/Comunicação

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

Rápida detecção de genes Van em *swabs* retais por PCR *real-time* na região sul do Brasil

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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 polimerase *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.

Infections due to vancomycin resistant enterococci (VRE) are a problem in hospitals worldwide[1] and are mostly mediated by genes vanA and vanB. In Brazil, vancomycin-resistant *Enterococcus faecium* and *E. faecalis* are disseminated in hospitals in different regions[2,3].

Recognition of colonized patients represents a crucial step in controlling the dissemination of these organisms and is a laboratory-based strategy[4]. Culture of rectal swabs is traditionally used to identify VRE colonized individuals and the turnaround time of this test typically exceeds 48h. The use of protocols that include molecular methods may contribute to reducing the time necessary to obtain results, thus providing the possibility of an intervention in a more convenient timeframe.

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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)[5-8]. 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 Enterococcosel™ agar (BBL, USA) supplemented with 6μg of vancomycin per mL and incubated at 35ºC 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[9].

Following the conventional procedure for VRE screening, rectal swabs were inoculated into Brain Heart Infusion (BHI, Difco, USA) broth, supplemented with gentamicin (8μg/mL) plus vancomycin (6μg/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'- AGCTGTACTCTCGCCGGATA-3' and VAN-A2-LC: 5'-CGCAGCCTACAAAAGGGATA-3'; VAN-B1-LC: 5'-AGGCGAGGACGCTTACCTAC-3' and VAN-B2-LC: 5'-AGGCGAGGACGCTTACCTAC-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: 79ºC, VanB: 83ºC).

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 purpose[1]. 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 most effective approach[5-8].

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[7]. 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 studies[10]. 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 predictive value		Negative predictive value	
	n	%	n	%	n	%	n	%
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.

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REFERENCES

1. Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). Diagn Microbiol Infect Dis 2004; 50:59-69.
2. d'Azevedo PA, Furtado GH, Medeiros EA, Santiago KA, Silbert S, Pignatari AC. Molecular characterization of vancomycin-resistant enterococci strains eight years apart from its first isolation in São Paulo, Brazil. Rev Inst Med Trop Sao Paulo 2008; 50:195-198.
3. Titze-de-Almeida R, Van Belkum A, Felipe MS, Zanella RC, Top J, Willems RJ. Multilocus sequence typing of hospital-associated *Enterococcus faecium* from Brazil reveals their unique evolutionary history. Microb Drug Resist 2006; 12:121.
4. Centers for Disease Control and Prevention. Recommendations for preventing the spread of vancomycin resistance. Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). Morb Mortal Wkly Rep Recomm Rep 1995; 44:1-13.
5. Drews SJ, Johnson G, Gharabaghi F, Roscoe M, Matlow A, Tellier R, et al. A 24-hour screening protocol for identification of vancomycin-resistant *Enterococcus faecium*. J Clin Microbiol 2006; 44:1578-1580.
6. Palladino SI, Kay D, Flexman JP, Boehm I, Costa AMG, Lambert EJ, et al. Rapid detection of vanA and vanB genes directly from clinical specimens and enrichment broths by real-time multiplex PCR assay. J Clin Microbiol 2003; 41:2483-2486.
7. Sloan LM, Uhl JR, Vetter EA, Schlech CD, Harmsen WS, Manahan J, et al. Comparison of the Roche LightCycler *vanA/vanB* detection assay and culture for detection of vancomycin-resistant enterococci from perianal swabs. J Clin Microbiol 2004; 42:2636-2643.
8. Stampler PD, Cai M, Lema C, Eskey K, Carroll KC. Comparison of the BD GeneOhm vanR assay to culture for identification of vancomycin-resistant enterococci in rectal and stool specimens. J Clin Microbiol 2007; 45:3360-3365.
9. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement. Approved standard M100:S18. Wayne (Pa): Clinical Laboratory Standards Institute; 2008.
10. Camargo IL, Barth AL, Pilger K, Seligman BG, Machado AR, Darini AL. *Enterococcus gallinarum* carrying the van A gene cluster: first report in Brazil. Braz J Med Biol Res 2004; 37:1669-1671.