Evaluation of the EVIGENE VRE Detection kit for detection of \textit{vanA} and \textit{vanB} genes in vancomycin-resistant enterococci

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The aim of this study was to evaluate the performance of the EVIGENE VRE Detection kit and compare it with PCR, considered the gold standard for detection of vancomycin-resistant enterococci (VRE). The correlation between the MIC values of vancomycin and teicoplanin using the epsilon test was also determined. In the EVIGENE VRE Detection kit, DNA probes specific for bacterial target DNA sequences are bound to microwell plates. A hundred and ten VRE (104 \textit{Enterococcus faecium} and six \textit{Enterococcus faecalis}) and 45 vancomycin-susceptible \textit{E. faecium} were tested. All VRE strains were found to be positive for the \textit{vanA} genotype using the EVIGENE VRE Detection kit. All results obtained with the EVIGENE VRE Detection kit were confirmed by PCR. MIC results for the strains also correlated highly with the PCR and kit results. The EVIGENE VRE Detection kit should be used in preference to other methods for detecting resistance genes in all strains, since it is less time-consuming, does not require the handling of hazardous chemicals and has the same specificity as PCR.

**INTRODUCTION**

In the last decade, the role of enterococci in serious clinical and nosocomial infections has been increasing. The spread of vancomycin-resistant enterococci (VRE) has become an important clinical concern, and VRE are now accepted as an emerging problem in hospitals worldwide (Palladino \textit{et al.}, 2003).

In enterococci, two principal phenotypes of acquired vancomycin resistance have been described, VanA and VanB (Bell \textit{et al.}, 1998). Strains with a VanA phenotype possess high-level resistance to both vancomycin and teicoplanin, whereas strains with a VanB phenotype possess only moderate to high levels of vancomycin resistance (Patel \textit{et al.}, 1997).

In the USA, about 70\% of vancomycin-resistant isolates currently show the VanA phenotype, while about 25\% show the VanB phenotype (Moellering, 1998). In Europe, although the incidence of infection caused by VRE is very low, except in the UK, VanA phenotype VRE are widespread among hospitalized and non-hospitalized patients (Descheemaeker \textit{et al.}, 2000). In Turkey, there have been only two studies concerning enterococci with the VanA phenotype. An outbreak due to \textit{Enterococcus faecium} carrying the \textit{vanA} gene was reported in a study carried out in Antalya (Colak \textit{et al.}, 2002) and \textit{vanA}-genotype enterococci have been described in a paediatric hospital in Ankara (Kilic \textit{et al.}, 2004).

The rapid identification of VRE in both colonized and infected patients is important for appropriate antimicrobial treatment and prevention of VRE (Hospital Infection Control Practices Advisory Committee, 1995).

The aim of this study was to evaluate the performance of the EVIGENE VRE Detection kit (Statens Serum Institute; (http://www.ssi.dk/graphics/dk/produktur/diagnostika/VRE.Brochure.PDF) based on the detection of VRE genes using DNA probe hybridization. The results obtained using this kit were compared with PCR results. Furthermore, epsilon tests (Etests) were performed for the determination of vancomycin and teicoplanin MICs.

**METHODS**

**Bacterial isolates.** A total of 155 enterococci strains [110 VRE (104 \textit{E. faecium} and six \textit{Enterococcus faecalis}) and 45 vancomycin-susceptible \textit{E. faecium}] isolated from 155 different patients and obtained from three referral training centres in different cities in Turkey were included in this study (Table 1). Strains were identified to species level by conventional
Table 1. Source of clinical enterococci isolates

<table>
<thead>
<tr>
<th>Source</th>
<th>E. faecium (n = 149)</th>
<th>E. faecalis (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>35</td>
<td>–</td>
</tr>
<tr>
<td>Rectal swab or stool</td>
<td>98</td>
<td>6</td>
</tr>
<tr>
<td>Wound</td>
<td>13</td>
<td>–</td>
</tr>
<tr>
<td>Catheter tip</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>1</td>
<td>–</td>
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</table>

Susceptibility tests. Etest MICs of vancomycin and teicoplanin were determined following the manufacturer’s instructions (AB-Biodisk) and interpreted using criteria set out by the National Committee for Clinical Laboratory Standards (NCCLS, 2004).

PCR procedure. Template DNA was prepared as described by Bell et al. (1998). The primers 5’-GGGAAAAGCACAATTGC-3’ and 5’-GGAACCCCGTTA-3’ (giving a 732 bp product) were used to detect the vanA gene in all of the Enterococcus strains ( Dutka-Malen et al., 1995). We designed vanB primers (5’-TACCTACCTCTGGTCTTGTGGAAGCC-3’ and 5’-GTTTTTTCGCGCTCGTTTTCGATG-3’, giving a 263 bp product) and vanC-2 primers [5’-GTTTCTTATAGCCAAATGACCG(T/G)T-G3’ and 5’-GTCAACAGCCGACGACTGAA-3’, giving a 192 bp product] with reference to the sequences deposited in GenBank under accession numbers L06138 and L29638, respectively. PCR was performed in a volume of 50 μl containing 1:2 Taq DNA polymerase (Fermentas), 0.1–1.0 ng template DNA (measured using a Jenway 6405 UV/Vis spectrophotometer), 0.2 mM dNTPs (Fermentas), 2.5 mM MgCl2 and 50 pmol each primer. PCR cycle conditions were 94°C for 1 min, 50°C for vanA or 60°C for vanB and vanC-2 for 2 min, and 72°C for 2 min for 35 cycles, with a final elongation step of 5 min at 72°C. Amplified DNA was electrophoresed on a 1.5%-agarose gel, stained with ethidium bromide and visualized under UV illumination (Gel Doc 2000; Bio-Rad). Two PCRs were performed for each sample.

EVIGENE VRE Detection kit. Tests were performed according to the manufacturer’s instructions (http://www.sxi.dk). The kit includes 24 break-apart tests, in a 96-microwell format, and contains seven reagents (A–G). Reagents A and B are used for bacterial lysis, reagent C is a hybridization buffer containing the biotinylated detection probes and reagents D–G are used for visualization of the captured genes. Each test consists of four wells: a positive control 16S rRNA gene, a negative control, and vanA and vanB wells. The 16S rRNA gene and vanA and vanB wells are coated with capture probes for the respective genes. Approximately 10 μl bacteria grown overnight on Columbia agar plates (Columbia plus 5% horse blood; Merck) was used for this method. The results of the tests could be evaluated after 3.5 h, either visually or using an ELISA reader. A test was considered positive when a yellow colour was clearly visible in the well, with a cut-off value equal to an A405 of 0.8.

RESULTS AND DISCUSSION

The susceptibility results of vancomycin and teicoplanin are shown in Table 2. A 732 bp PCR product was obtained from 110 enterococci (104 E. faecium and six E. faecalis) (Fig. 1a). vanB and vanC-2 genes were not detected in any of the isolates (Fig. 1b and c, respectively). All enterococci strains were tested with the EVIGENE VRE Detection kit. The 110 VRE isolates were vanA positive (median A405 = 1.962, range 1.192–2.529) and vanB negative (median A405 = 0.519, range 0.462–0.581), while the 45 vancomycin-susceptible E. faecium isolates were vanA and vanB negative (median A405 = 0.521, range 0.452–0.602; median A405 = 0.532, range 0.482–0.599, respectively). Cross-hybridization to the other genes was not observed. The isolates found to be vanA positive with the EVIGENE VRE Detection kit were in complete accordance with the PCR results. The specificity and sensitivity was 100% when compared with the results of PCR. The results obtained with the EVIGENE VRE Detection kit are summarized in Figs 2 and 3.

In many countries, hospitals now have surveillance programmes for VRE. However, most use culture-based phenotypic methods, which have inherent limitations (Rogers et al., 1999). Phenotypic methods are limited in their ability to detect low-level glycopeptide resistance and to determine the difference between van types (Palladino et al., 2003; Luh et al., 2000; Sahm et al., 1997). Also, the phenotypic susceptibility tests require a 24–48 h period of incubation following isolation of a strain. As a result, routine phenotypic tests for VRE are unreliable in certain situations and time consuming (Jorgensen & Ferraro, 2000). The EVIGENE VRE Detection kit offers rapid (3.5 h) and direct detection of the vanA and vanB genes from a pure culture of enterococci. The kit cannot differentiate other vancomycin-resistance genotypes (vanC, vanD and vanE), but this does not seem to be an important problem since these do not cause life-threatening infections. The vanA and vanB genotypes are currently the most prevalent in the world and cause serious infections.

Table 2. Genotypes and in vitro activity of two antimicrobial agents against enterococci

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Genotype determined by PCR</th>
<th>Genotype determined by kit</th>
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<tbody>
<tr>
<td></td>
<td>Vancomycin</td>
<td>Teicoplanin</td>
</tr>
<tr>
<td>E. faecium (n = 104)</td>
<td>≥256 (n = 104)</td>
<td>≥32 (n = 74), 12 to &lt; 32 (n = 30)</td>
</tr>
<tr>
<td>E. faecium (n = 45)</td>
<td>≤4 (n = 45)</td>
<td>≤8 (n = 45)</td>
</tr>
<tr>
<td>E. faecalis (n = 6)</td>
<td>≥256 (n = 6)</td>
<td>≥32 (n = 1), 12 to &lt; 32 (n = 5)</td>
</tr>
</tbody>
</table>
Many microbiology laboratories have recently introduced PCR for detection and confirmation of the presence of VRE (Satake et al., 1997). Unfortunately, the high sensitivity of PCR and other DNA amplification methods carries the risk of sample cross-contamination (Dutka-Malen et al., 1995).

In addition, the amplified products are commonly detected by agarose gel electrophoresis, which employs hazardous chemicals (ethidium bromide), and may be complicated by non-specific bands, thus resulting in subjectivity in reading results (Petrich et al., 1999). Nevertheless, the total time required for PCR analysis, including sample preparation and gel electrophoresis, is about 3.5 h (Kanchana et al., 2000).

The EVIGENE VRE Detection kit does not contain hazardous chemicals and is easy to evaluate. To our knowledge, there has been no previous report of EVIGENE VRE Detection kit results in PubMed.

Fig. 1. Agarose gel electrophoresis of PCR products. (a) PCR of the vanA gene with a product size of 732 bp. Lanes: 1, E. faecium B7641; 2, vancomycin-resistant E. faecium (test isolate); 3, E. faecalis (test isolate); 4, vancomycin-susceptible E. faecium (test isolate); 5, E. faecium ATCC 6057 (wild-type control). (b) PCR of the vanB gene with a product size of 263 bp. Lanes: 1, E. faecalis V583; 2–5 as in (a). (c) PCR of the vanC-2 gene with a product size of 192 bp. Lanes: 1, E. casseliflavus ATCC 25788; 2–5 as in (a). SM, DNA size marker.

Fig. 2. Median A_{405} results obtained with the EVIGENE VRE Detection kit for 155 enterococci. 16S rRNA, positive-control well; Neg., negative-control well; VanA and VanB, signals obtained using the probes. Black bars, VRE strains; white bars, vancomycin-susceptible E. faecium.

Fig. 3. Results obtained with the EVIGENE VRE Detection kit. Lanes: 1, E. faecium B7641 (vanA); 2, E. faecalis V583 (vanB); 3, E. casseliflavus ATCC 25788 (vanC-2); 4, vancomycin-resistant E. faecium (test isolate); 5, E. faecalis (test isolate); 6, vancomycin-susceptible E. faecium (test isolate); 7, E. faecium ATCC 6057 (wild-type control). Rows: A, 16S rRNA (positive-control well); B, negative-control well; C, vanA well; D, vanB well.
In summary, the EVIGENE VRE Detection kit detected the vancomycin-resistance \textit{vanA} gene in all PCR-positive strains. This test should be used in preference to phenotypic methods since it is quicker and does not require experienced staff or hazardous chemicals.

REFERENCES


