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Evaluation of the Xpert vanA/vanB Assay Using Enriched Inoculated Broths for Direct Detection of vanB Vancomycin-Resistant Enterococci


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Rapid and accurate detection of VRE (vancomycin-resistant enterococci) is required for adequate antimicrobial treatment and infection prevention measures. Previous studies using PCR for the detection of VRE, including Cepheid’s Xpert vanA/vanB assay, reported accurate detection of vanA VRE; however, many false-positive results were found for vanB VRE. This is mainly due to nonenterococcal vanB genes, which can be found in the gut flora. Our goal was to optimize the rapid and accurate detection of vanB VRE and to improve the positive predictive value (PPV) by limiting false-positive results. We evaluated the use of the Xpert vanA/vanB assay on rectal swabs and on enriched inoculated broths for the detection of vanB VRE. By adjusting the cycle threshold (C_T) cutoff value to ≤25 for positivity by PCR on enriched broths, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 96.9%, 100%, 100%, and 99.5% for vanB VRE, respectively. As shown in this study, C_T values of ≤25 acquired from enriched broths can be considered true positive. For broths with C_T values between 25 and 30, we recommend confirming the results by culture. C_T values of >30 appeared to be true negative. In conclusion, this study shows that the Cepheid’s Xpert vanA/vanB assay performed on enriched inoculated broths with an adjusted cutoff C_T value is a useful and rapid tool for the detection of vanB VRE.

Vancomycin-resistant enterococci (VRE) have emerged as an important nosocomial problem worldwide. The rise of VRE is mostly due to Enterococcus faecium, with vanA and vanB being the two clinically most important genotypes (1). VanA-type resistance is induced by teicoplanin and vancomycin, causing resistance to both antibiotics. In contrast, VanB-type resistance is induced only by vancomycin, resulting in isolates that have variable levels of vancomycin resistance but are still susceptible to teicoplanin (2).

Rapid and accurate detection of VRE is required for adequate antimicrobial treatment and infection prevention measures. Culture-based methods to detect VRE are often time-consuming and take several days to complete (2 to 5 days). As part of the infection control measures that have to be taken by the hospital, especially during outbreaks, these time-consuming methods have a high economic impact (3). Several studies evaluated PCR-based methods for rapid detection of VRE, including the Cepheid Xpert vanA/vanB assay (4–7). This assay runs on the Cepheid GeneXpert system, a fully automated processor that combines DNA extraction, real-time PCR amplification, and detection, providing results within an hour. PCR-based methods are highly sensitive and specific for the detection of vanA VRE (7). However, for vanB VRE, many false-positive results are reported, mainly due to nonenterococcal vanB genes, which can be found in the gut, especially in anaerobic bacteria like Clostridium species (8–11). Therefore, positive vanB VRE results still need to be confirmed by culture. An additional problem is that VanB-type resistance is sometimes difficult to detect, since the vancomycin MIC can be below the antimicrobial susceptibility breakpoint of ≤4 mg/liter defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (12–14).

In our hospital, VRE screening is performed for patients coming from foreign hospitals, for those in ICU wards, and in cases of an unexpected VRE observation, e.g., if VRE is found in clinical specimens from epidemiologically linked patients. Isolation precautions are applied to patients coming from foreign hospitals at admission until patient samples are negative. During a suspected VRE outbreak, patients are grouped into cohorts and screened on a regular basis. Prior to this study, VRE detection was performed on enriched inoculated broths with a conventional gel-based PCR. However, many false-positive results were obtained with this technique.

In March and April 2013, our hospital faced an outbreak of vanB VRE. During this outbreak, we used and evaluated the Xpert vanA/vanB assay on rectal swabs and on enriched inoculated broths. Our goal was to optimize the rapid and accurate detection of vanB VRE and to improve the positive predictive value (PPV) by limiting false-positive results.

MATERIALS AND METHODS

The University Medical Center Groningen is a 1,300-bed tertiary care center. During an outbreak of vanB VRE in March and April 2013, rectal swabs (Copan ESwabs) were collected from hospitalized patients at the relevant wards for VRE testing.

Lab study design. In total, 235 ESwabs from 91 patients were used. The Xpert vanA/vanB assay was directly performed on 100 μl ESwab medium and on enriched inoculated broths after 24 h of incubation. For the latter, 9 ml of brain heart infusion (BHI) broth containing 16 mg/liter amoxicillin, 20 mg/liter amphotericin B, 20 mg/liter aztreonam, and 20
mg/liter colistin was inoculated with 400 µl ESwab medium and incubated at 35°C for 24 h. Amoxicillin was used, since VRE outbreaks are typically caused by amoxicillin-resistant enterococci (ARE) that have acquired resistance to vancomycin (15, 16). Moreover, amoxicillin limits the growth of amoxicillin-sensitive anaerobic bacteria, like Clostridium species, which are some of the most relevant species that may contain vanB genes (8, 17, 17). Vancomycin was not added to the broth, as this would hamper the detection of vanB VRE exhibiting low vancomycin MICs (12, 14). From the broths, 100 µl was used in the Xpert vanA/vanB assay, and 10 µl of broth was subcultured on VRE Brilliance agars (Oxoid). Agars were incubated at 35°C and examined after 24 to 48 h. Blue colonies suspected for enterococci were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker). Confirmed Enterococcus species were tested for antibiotic susceptibility using Vitek2 (bioMérieux). The MIC clinical breakpoints defined by the EUCAST for Enterococcus spp. are as follows: for vancomycin, susceptible, ≤4 mg/liter, and resistant, >4 mg/liter; for teicoplanin, susceptible, ≤2 mg/liter, and resistant, >2 mg/liter (17). Colonies were subsequently analyzed in the Xpert vanA/vanB assay. When the Xpert vanA/vanB assay was negative, 3 to 5 more colonies were tested (Fig. 1).

The Cepheid GeneXpert system. The Xpert vanA/vanB assay was performed on three different sources, as depicted in Fig. 1. For ESwab medium as well as for enriched inoculated broths, 100 µl was added to the elution buffer, and the mixture was vortexed for 10 s and transferred into the Xpert vanA/vanB cartridge. For isolates on VRE Brilliance agar, a suspension of 1 or 2 bacterial colonies was made using 1 ml Milli-Q water (Sigma-Aldrich), vortexed for 10 s, and then diluted 1:500 in Milli-Q water. Subsequently, 75 µl of the diluted sample was added to the elution buffer and transferred to the Xpert vanA/vanB cartridge. The amounts used for the Xpert vanA/vanB assay were those recommended by the manufacturers and/or validated in our laboratory. Further procedures were performed according to the manufacturers’ guidelines (Xpert vanA/vanB; guideline 710001616). According to these guidelines, cycle threshold (CT) values of ≤36 are considered positive, whereas CT values of >36 are considered negative. A CT value of 0 for vanA and vanB indicates no amplification and is considered negative if the internal control present in the assay is valid.

Resolution of discordant results. As mentioned before, VRE detection is difficult, and different detection methods are used. The use of a direct rectal swab culture method for the detection of VRE colonization

FIG 1 Workflow of the study, including definitions of negative and positive VRE in bold (gold standard). The numbers 1, 2, and 3 indicate the three sources used in the Cepheid Xpert vanA/vanB assay.
shows a high rate of false-negative results (18). Culture of rectal samples after broth enrichment followed by species identification and susceptibility testing is most sensitive for detecting VRE (19). Therefore, we defined a sample as true positive for VRE when the Xpert assay performed on the isolate from the VRE Brillance agar, after broth enrichment, was positive (our gold standard). A sample was considered true negative for VRE in the case of the following results: (i) observation of no growth on VRE Brillance agar after broth enrichment, (ii) growth of species other than Enterococcus species on the VRE Brillance agar, or (iii) growth of Enterococcus species on VRE Brillance agar but a negative Xpert assay performed on the isolate (Fig. 1).

The new algorithm. Based on the CT values acquired from ESwab medium compared to those acquired from the enriched inoculated broths, we defined a new cutoff value for positivity by PCR on enriched broths. Broths with CT values of ≤25 were considered to be true positive for vanA VRE, whereas broths with CT values between 25 and 30 required confirmation by culture. Samples with CT values of 0 (no amplification) or >30 were considered negative. To test our new algorithm, we prospectively evaluated 112 enriched inoculated broths from routine screenings.

**Statistical methods.** Sensitivity, specificity, PPV, and negative predictive values (NPV) were calculated for the results of the Xpert assay performed directly on ESwab medium as well as on enriched inoculated broths. The 95% confidence intervals were calculated using the Wilson 95% confidence interval, including continuity correction (20).

**RESULTS**

Out of 235 ESwabs, 157 were negative in the Xpert vanA/vanB assay and confirmed to be true negative for VRE according to our definitions (Table 1). In these 235 ESwabs, no vanA VRE was found. A total of 78 ESwabs were vanB VRE positive according to the assay, of which 32 were confirmed to be true positive for vanB VRE according to our definition. Moreover, all 32 isolates were identified as E. faecium and had a typical VanB phenotype by Vitek2 susceptibility testing. The MICs for these 32 isolates ranged from 8 mg/liter to >32 mg/liter for vancomycin; all teicoplanin MICs were <0.5 mg/liter. The other 46 ESwabs were positive according to the assay, but no VRE could be confirmed using our gold standard, and these were considered false positive. Therefore, the Xpert assay on ESwabs resulted in a sensitivity, specificity, PPV, and NPV of 100%, 77.3%, 41%, and 100%, respectively (Table 2).

Using the Xpert assay on enriched broths resulted in a decrease of CT values for the majority (80.6%) of true-positive cases compared to their CT values obtained directly from ESwabs. For true-negative cases, the opposite was observed for 94.7% of the samples (Fig. 2). Because of the observed decline in CT values of the broths, we adjusted the cutoff value for PCR positivity of the Xpert assay on broth to ≈25. Sensitivity, specificity, PPV, and NPV were recalculated and were 96.9%, 100%, 100%, and 99.5%, respectively (Table 1 and 2). By using a cutoff value of ≤25, no false-positive results were found; however, one true-positive VRE was missed (CT value of 25.9). Therefore, we defined a “gray zone” for samples with a CT value between 25 and 30 that require confirmation by culture. In this case, patients were not placed in cohorts with VRE-positive persons until these samples were confirmed by culture. CT values of >30 were considered to be true negative. Importantly, our algorithm was prospectively tested using 112 enriched inoculated broth samples. We found 80 true-negative samples, 31 true-positive samples, and one sample with a CT value of 28.4 which required confirmation. The new algorithm resulted in sensitivity, specificity, PPV, and NPV that were all 100%.

**DISCUSSION**

In this study, we evaluated the use of the Xpert vanA/vanB assay on rectal swab specimens and on enriched inoculated broths for the detection of vanB VRE. By using enriched broths combined with a new CT cutoff value of ≤25 for PCR positivity, the PPV for VRE detection increased from 41% to 100%. As shown in this study, CT values of ≤25 acquired from enriched broth can be considered true positive. For broth with CT values between 25 and 30, we recommend confirming the result by culture. CT values of >30 appeared to be true negative.

VRE detection remains difficult, and the question of the best method to be used for the most reliable results remains open for discussion. We are aware of the fact that use of feces is superior to use of rectal swabs, which were used in this study. However, use of feces is less practical for the clinicians during an outbreak screening. Some studies consider PCR-positive specimens to be true positive, whereas broths with CT values between 25 and 30 required confirmation. The new algorithm resulted in sensitivity, specificity, PPV, and NPV that were all 100%.

**TABLE 1** Xpert vanA/vanB assay results with ESwabs and inoculated enriched broths in relation to true VRE positivity and negativitya

<table>
<thead>
<tr>
<th>CT values</th>
<th>No. of samples</th>
<th>ESwabs (CTa ≤ 36)</th>
<th>Inoculated enriched broths (CTa ≤ 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTa ≤ 36</td>
<td></td>
<td>VRE positive</td>
<td>VRE negative</td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>46</td>
<td>78</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>157</td>
<td>157</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>203</td>
<td>235</td>
</tr>
</tbody>
</table>

a See Materials and Methods and Fig. 1 for definitions.

**TABLE 2** Sensitivity, specificity, PPV, and NPV of ESwab and inoculated enriched broth used in the Cepheid Xpert vanA/vanB PCRa

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESwab (CTa ≤ 36)</td>
<td>100 (86.7–100)</td>
<td>77.3 (70.8–82.8)</td>
<td>41 (30.2–52.8)</td>
<td>100 (97.2–100)</td>
</tr>
<tr>
<td>Inoculated enriched broth (CTa ≤ 25)</td>
<td>96.9 (82–99.8)</td>
<td>100 (97.7–100)</td>
<td>100 (86.3–100)</td>
<td>99.5 (96.9–100)</td>
</tr>
</tbody>
</table>

a Values in parentheses are 95% confidence intervals.
ever, as noted earlier, the majority of VRE outbreaks are typically caused by ARE that acquired resistance to vancomycin (15, 16).

All \textit{vanB} VRE isolates found in this study had a typical VanB phenotype, as determined by Vitek2. Remarkably, in this study no \textit{vanB} VRE exhibiting low vancomycin MICs were detected, although these strains have been found in our hospital in past years. Since no vancomycin was added to the broth, we are convinced that detection of these \textit{vanB} VRE strains were adequately performed. In addition, we observed that these strains grow on VRE Brilliance agars, as was also shown by others (12).

As a consequence, when the Xpert assay is performed on enriched broths instead of on direct rectal specimens, the first PCR results will follow 24 h later due to the extra incubation step. On the other hand, by using this method the PPV increases from 41% to 100%, which is essential for right decision making with respect to infection prevention. To control an outbreak, it is crucial to group true-negative patients apart from true-positive patients. An ongoing outbreak might require closure of the ward, which has a high financial impact and subsequently an enormous impact on patient care. Samples with \textit{C}_{T} values between 25 and 30 will take another 24 to 48 h, though only a minority of samples in our study had results among these values (0.6% of all samples). Therefore, the use of the Cepheid Xpert \textit{vanA/vanB} assay on inoculated enriched broths with an adjusted \textit{C}_{T} value for PCR positivity can be considered a useful and rapid tool for the detection of \textit{vanB} VRE.

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REFERENCES


FIG 2 Dynamics between \textit{C}_{T} values acquired by performing the Xpert \textit{vanA/vanB} assay directly on ESwabs (left) and on enriched inoculated broths (right). One line represents one sample. Blue lines represent samples with confirmed true-negative VRE cultures. Green lines represent samples with confirmed true-positive VRE cultures. The red line indicates the cutoff \textit{C}_{T} value for PCR positivity used by the Cepheid GeneXpert system (\textit{=36}). The purple line indicates the new cutoff \textit{C}_{T} value for PCR positivity (\textit{=25}). In this figure \textit{C}_{T} values of 45 indicate that no amplification was detected (negative test). * See Materials and Methods and Fig. 1 for definitions.


