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Comparison of direct cultivation on a selective solid medium, polymerase chain reaction from an enrichment broth, and the BD GeneOhm™ VanR Assay for identification of vancomycin-resistant enterococci in screening specimens

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Abstract

Fast and reliable diagnostics of vancomycin-resistant enterococci (VRE) is an important prerequisite for containing VRE transmission rates and controlling VRE outbreaks among hospital patients. The BD GeneOhm™ VanR Assay (Becton Dickinson Diagnostics, Erembodegem, Belgium) is a real-time polymerase chain reaction (PCR) assay for screening perianal/rectal samples for the presence of vanA *or* vanB *genes that can be associated with VRE. A set of 51 reference strains (*vanA–G *genotypes) were correctly identified. Performance of the assay was evaluated and compared with culture-based methods and subsequent PCR analysis in 2 university hospitals with a different VRE prevalence. A total of 1786 samples were analyzed. With the use of the BD GeneOhm™ VanR Assay, 88 of 102* vanA*-positive specimens, 62 of 67* vanB*-positive specimens, 3 of 4* vanA- *and* vanB*-positive specimens, and 1403 of 1613 negative specimens were correctly identified. The overall sensitivity was 93.1%; the specificity was 87.0% mainly due to false-positive* vanB *results. Results did not differ between study institutions.*

1. Introduction

Multiresistant pathogens are an increasing threat in the nosocomial setting. Colonization often precedes subsequent infection; consequently, “colonization pressure” is a critical term and unique for each kind of pathogen ([Bonten et al., 1998] and [Donskey, 2004]). For vancomycin-resistant enterococci, the ratio of colonization to infection is quite high, meaning a single patient with a vancomycin-resistant enterococci (VRE) infection corresponds to a larger number of colonized persons surrounding the index patient and the more patients are colonized, the higher is the risk of an increasing number of infected patients ([de Regt et al., 2008] and [Olivier et al., 2008]). Effective infection control and prevention measures are capable of reducing the colonization and transmission rates and thus correspondingly the infection rate. Therefore, early recognition and isolation of VRE colonized patients are critical steps in reducing VRE infections and terminating VRE outbreaks ([Aumeran et al., 2008] and [Deplano et al., 2007]). Various commercial phenotypic and genotypic assays are available for VRE screening differing in sensitivity and specificity ([Bourdon et al., 2010], [Grabsch et al., 2008], [Ledeboer et al., 2007], [Stamper et al., 2007], [Stamper et al., 2010] and [Young et al., 2007]). In general, genotypic assays may be advantageous over phenotypic tests being much faster and capable of circumventing problems associated with a sometimes low expression of *vanA*- and *vanB*-type resistance phenotypes ([Naas et al., 2005], [Stamper et al., 2007] and [Song et al., 2008]).

In recent years, VRE prevalence rates increased in many European countries ([Brown et al., 2008], [Henard et al., 2011], [Sagel et al., 2008], [Soderblom et al., 2010], [Theilacker et al.,

2009] and [Werner et al., 2008]). This has led to increased interest in screening of patients for colonization as well as to increased interest in methods used for a fast, sensitive, and reliable VRE detection (Vonberg et al., 2007). Rapid screening methods for VRE using direct polymerase chain reaction (PCR) from rectal swabs or stool have been described ([Palladino et al., 2003], [Petrich et al., 1999] and [Satake et al., 1997]); some of these assays are commercially available ([Bourdon et al., 2010], [Stamper et al., 2007], [Sloan et al., 2004] and [Usacheva et al., 2010]). These tests have been evaluated in a few studies performed, and excellent sensitivity and specificity for detecting *vanA* enterococci were shown, and a low specificity due to comparably high rates of supposed false-positive *vanB* samples appeared as a problem ([Stamper et al., 2007] and [Sloan et al., 2004]). Some investigators linked this phenomenon to commensal bacteria of the fecal flora that carry the *vanB* gene ([Ballard et al., 2005a], [Ballard et al., 2005b], [Domingo et al., 2005], [Grabsch et al., 2008], [Graham et al., 2008], [Stamper et al., 2007] and [Sloan et al., 2004]), but it is not known whether this phenomenon is equally distributed among different parts of the world.

In our patient population, we have previously seen percentages of >20% of VRE ([Kresken et al., 2009] and [Theilacker et al., 2009]) and thus were interested in the performance of a PCR-based screening method in this population. In addition, VRE prevalence varies among closely localized institutions in southwestern Germany, indicating a different epidemiologic situation, which may also influence the performance of the assay ([Meyer et al., 2006] and [Sagel et al., 2008]).

Here we demonstrate the performance of the BD GeneOhm™ VanR Assay in detecting *vanA* and *vanB* genes which can be associated with VRE. We determined the sensitivity and specificity of the assay to identify in vitro reference and well-characterized test and reference strains. Intralaboratory comparability of results was determined by performing a proficiency test with 20 representative strains in a blinded manner. In vivo performance of the BD GeneOhm™ VanR Assay was compared with direct cultivation on selective solid agar media and cultivation in an enrichment broth each followed by PCR for VRE screening in 2 patient populations at 2 university hospitals with different VRE prevalence.

2. Materials and methods

2.1. Reference strain collection and in vitro study

Strains included in the in vitro study were *Enterococcus* spp. reference and test strains. They included reference strains for the *vanA–G* genotypes encoding vancomycin resistance in enterococci and their respective subtypes (e.g., *vanB*-1 to *vanB*-3; Table 1). The reference and test strains were characterized by standard PCR for the presence of *van* genes (Depardieu et al., 2004). They were typed by molecular methods (multilocus sequence typing [MLST], Pulsed field gel electrophoresis) to demonstrate unique and different strain types. The methods and most of the involved isolates were already described in previous articles (see Table 1 for details). Twenty well-characterized and clonally different vancomycin-resistant and susceptible *E. faecium* and *E. faecalis* isolates were chosen for an in vitro proficiency test performed at 3 different medical–microbiological laboratories, 2 of which later on participated in the in vivo study. Characteristics of strains were not communicated to the partners (blinded study).

2.2. In vivo study — patients and specimens

The study was performed in 2 different institutions. Both institutions are university hospitals each with more than 1400 beds, but they differ significantly in their VRE prevalence (Table 2). Both institutions had experienced outbreaks of VRE, and infection control programs were harmonized (von Baum et al., 2006). VRE screening was performed by rectal swabs, which were taken in accordance with the institutions' infection control program. Swabs were obtained by nursing staff using solid Amies medium swabs (uni-Ter Amies CLR, Medical Wire & Equipment Co. (Bath) Ltd. Corsham, England, or MEUS, Srl, Piove di Sacco, Italy) and solid Amies medium swabs without charcoal (COPAN innovation Venturi Transystem, Copan Italia Spa, Breccia, Italy) at institutions 1 and 2, respectively. The study targeted approximately a total of 150 positive specimens. To this extent, at least 1500 tests had been

performed including patients who were known carriers of VRE and who were sampled repetitively (on average 2 times) with a minimum of 1-week intervals between different tests.

2.3. Specimen processing

The BD GeneOhm™ VanR Assay is a real-time PCR assay (Smart Cycler®) configured to screen for VRE colonization in hospital patients by testing perianal and/or rectal swabs for *vanA* and *vanB* genes. In the in vitro study, the standard protocol (BD GeneOhm™ VanR Assay) was modified starting with isolated and freshly diluted cultures of enterococci equivalent to ca. 1/100 McFarland 0.5 (ca. 10^6 CFU/sample). A swab was dipped into the diluted sample, removed, and placed into the sample buffer tube (provided by the kit). The swab stem was broken and the sample buffer tube was closed. Samples were subsequently treated according to the standard protocol of the BD GeneOhm™ VanR Assay. For the in vivo test evaluation at the 2 hospital settings, the same swab was used to inoculate a Columbia blood agar (BD Diagnostics, Heidelberg, Germany) and a premanufactured Bile Esculin-Azide Agar plate (BEAV agar) with 6 µg/mL of vancomycin (BD Diagnostics). Then the tip of the swab was broken off into a sample buffer tube containing a Tris-EDTA sample preparation buffer, provided by the manufacturer of the BD GeneOhm™ VanR Assay. After the sample buffer tube was vortexed for 1 min, 500 µL of the solution was inoculated into the Enterococcosel™ broth (Becton-Dickinson, Germany) supplemented locally with 0.4% (4 µg/mL) vancomycin (Vancomycin Lederle, Riemser, Germany), and 50 µL of the solution was transferred into a BD GeneOhm™ VanR Lysis tube for further PCR processing. The remaining content of the sample preparation buffer was stored frozen at −20 °C.

2.4. Culture method

All primary culture plates and broths were incubated in ambient air at 36 ± 2 °C. Columbia blood agar was reviewed after 18 to 28 h of incubation. If there was lack of bacterial growths on blood agar, the specimen was rated as inadequate and the specimen was excluded from further analysis. Broths and BEAV plates were reviewed daily before being called negative after 2 days of incubation. Black discoloration or cloudiness in the Enterococcosel™ broth was considered a positive result. PCR was performed directly from positive broths. Brown to black colonies on BEAV plates were considered presumptively positive and tested by PCR if the broths remained negative. If PCR yielded positive results, the positive broth or the BEAV plate, respectively, was subcultured to a Columbia blood agar. Bacterial colonies with typical appearance were identified using the Vitek 2 system (BioMérieux, Nürtingen, Germany). The *van* genotype was confirmed by PCR from culture.

2.5. PCR Testing

An in-house multiplex real-time PCR was performed for detecting the *vanA* and the *vanB* genes based on primer pairs published recently ([Dutka-Malen et al., 1995a], [Dutka-Malen et al., 1995b] and [McGregor and Young, 2000]). DNA was extracted from broths by repeated centrifugation of 1.5 mL broth for 5 min at 13,000 rpm followed by washing of the pellet with aqua dest. Then the pellet was resuspended in 500 µL TE buffer with glass beads and heated for 15 min at 100 °C. After vigorous shaking for 15 min on a vortex device, the sample was centrifuged again for 5 min at 13,000 rpm. Five microliters of the supernatant was diluted in 500 µL aqua dest. and used for PCR.

DNA from colonies was extracted by homogenization of 3 to 4 colonies in 100 µL aqua ad inject, followed by heating for 15 min at 95 °C and then centrifuged for 5 min at 13,000 rpm. The supernatant was used for PCR.

Five microliters of supernatant from broth or 2.5 µL of supernatant from bacterial colonies was used for PCR. Primers used for *vanA*-gene detection were 5′-ATG AAT AGA ATA AAA GTT GCA ATA CT-3′ (forward) and 5′-GGC GAG AGT ACA GCT GAA TA-3′ (reverse) with a fluorescent hybridization probe (*vanA*: 5′-TET-CTC AGA GGA GCA TGA CGT ATC GGT-BHQI-3′), and primers used for *vanB*-gene detection were 5′-GGA CAA ATC ACT GGC CTA CAT TC-3′ (forward) and 5′-CGC CGA CAA TCA AAT CAT C-3′ (reverse) with a fluorescent hybridization probe (*vanB*: 5′-FAM-ACC TAC CCT GTC TTT GTG AAG CCG -BHQI-3′). As an internal control, the 16S-eubac-gene (Unibac) was

amplified using the Unibac primer (forward: 5′-TGG AGC ATG TGG TTT AAT TCG A-3′; reverse: 5′-TGC GGG ACT TAA CCC AAC A-3′) with a fluorescent hybridization probe (Unibac: 5′-TX Red-CAC GAG CTG ACG ACA (AG)CC ATG CA –BHQII-3′).

The PCR was performed in a SmartCycler® II device (Cepheid, Sunnyvale, CA, USA). Cycling conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 37 cycles of 15 s at 95 °C, 30 s at 56 °C, and 30 s at 72 °C. Amplification curves had to have a typical sigmoid form and had to exceed the threshold (calculated from values between cycles 5 and 10, plus 15-fold standard deviation) to be counted as positive. A *vanA*-positive *E. faecium* isolate and a *vanB*-positive *E. faecalis* isolate (both well-characterized clinical isolates) served as positive controls. Positive and negative controls were carried along with each test.

2.6. BD GeneOhm™ VanR Assay

After inoculation with the swab as mentioned previously, 50 µL of the sample buffer was transferred into a lysis tube containing glass beads. The lysis tube was vortexed for 5 min at high speed and centrifuged briefly. The sample was heated at 95 °C for 2 min and placed on ice. The master mix was reconstituted by diluting it with 225 µL of diluent and vortexing for 5–10 s. A control DNA tube was placed on ice and 225 µL of the sample buffer was added. The sample was vortexed shortly (5–10 s). SmartCycler® tubes were placed on the SmartCycler® cooling block and 25 µL of reconstituted Master Mix was added to each SmartCycler® tube. Of each lysed specimen, 3 µL was added to the previously filled SmartCycler® tubes and the tubes were closed. Finally, 3 µL of the reconstituted control DNA was added to 1 SmartCycler® tube (positive control) and 3 µL of uninoculated sample buffer was added to the last SmartCycler® tube (negative control). After all tubes were closed, they were centrifuged briefly and cooled on the cooling block until loading the instrument. With the use of the SmartCycler® device, a new run was created with the BD GeneOhm™ VanR Assay protocol. The reaction tubes were inserted into the I-CORE® modules of the SmartCycler® instrument and the lids were closed. The run was started according to the manufacturer's software and amplification protocol for the BD GeneOhm™ VanR Assay. The real-time PCR procedure includes positive and negative controls as well as an internal process control. Amplified products for *vanA* and *vanB* are detected via labelled probes. PCR conditions and data analysis are standardized and automatically reported by the assay software which makes the test user friendly and helps prevent user errors. Results are reported as follows: “POS” for detecting *vanA*; “Presumptive POS” for detecting *vanB*; “NEG” for no detection of *vanA/B*; “Positive” for a simultaneous detection of *vanA* and *vanB*; “Unresolved” and “ND” for process control inhibition or thermocycler failure, respectively; and “Valid/Invalid” for valid or invalid positive and/or negative control sample results. Samples with inhibition of the internal control were retested from the original frozen sample. The processed lysis tubes and SmartCycler® tubes, containing patient samples, were stored at −20 °C.

2.7. Subsequent analysis of ambiguous results

Samples in which the culture result and the BD GeneOhm™ VanR Assay result differed were tested again using the original frozen sample preparation buffer for culture and the lysate for the PCR. The test results from the first BD GeneOhm™ VanR Assay PCR and from the first culture approach were used for the primary analysis of the data.

2.8. Data analysis

Samples which grew at least 1 *vanA*- or *vanB* gene-positive isolate were determined to be VRE positive. The recovery rate on solid medium (BEAV agar) and in the Enterococcosel™ broth with 4 µg/mL vancomycin was compared to all culture-positive samples. The sum of all culture-positive samples served as the gold standard for the evaluation of the BD GeneOhm™ VanR Assay. The BD GeneOhm™ VanR Assay results were determined according to the manufacturer's specifications as negative, either *vanA* positive or *vanB* positive, or positive for both *vanA* and *vanB*, and compared to all culture-positive samples. Descriptive statistics were determined for different VRE genotypes and stratified by study institution. Prevalence, sensitivity, specificity, predictive values, and their confidence

intervals were calculated using Clinical Calculator 1 (http://faculty.vassar.edu/lowry/clin1.html, accessed 21/1/2011).

2.9. Multilocus sequence typing

PCRs amplifying the 7 loci used for MLST were done according to the reference MLST database (http://efaecium.mlst.net/). Sequencing reactions were performed according to the manufacturer's recommendations for cycle sequencing of PCR products (Applied Biosystems, Life Technologies Corp., Carlsbad, CA, USA). Sequence files were read, evaluated, aligned, and compared with the reference set of alleles using the sequencing software Lasergene 8.0 from DNASTAR (SeqMan 8.0; EditSeq 8.0, DNASTAR Inc., Madison, WI, USA), TraceEditPro v. 1.1.1 from Ridom (http://www.ridom.de), and via the official MLST webpage (http://efaecium.mlst.net/).

3. Results

3.1. In vitro study

Performance of the BD GeneOhm™ VanR Assay in identifying 51 reference and well-characterized test strains including *vanA*-type *E. faecium*, *vanA*-type *E. faecalis*, *vanB*-type *E. faecium*, *vanB*-type *E. faecalis*, *vanB*-type *E. durans*, *vanB*-type *E. mundtii*, *vanC-1* type *E. gallinarum*, *vanC-2* type *E. casseliflavus*, as well as of the *vanD*-, *vanE*-, and *vanG*-type *Enterococcus* reference isolates was assessed (Table 1). All strains of *vanA* and *vanB* genotypes were correctly identified irrespective of their corresponding species or clonal background (MLST type). All naturally vancomycin-resistant and intermediately resistant *E. gallinarum* and *E. casseliflavus* of the *vanC* genotypes revealed a negative test result (test result “NEG”; Table 1). A single *E. gallinarum* tested positive (POS) by the BD GeneOhm™ VanR Assay due to a *vanA* gene cluster acquired in addition to the intrinsic *vanC1* gene cluster. Isolates harbouring acquired non-*vanA/vanB van* genotypes, *vanE* to *vanG*, revealed a negative test result (Table 1). The latter would be categorized as VRE, too; however, the negative test results correspond to the test design which allows identification of *vanA* and *vanB* genotypes only based on complementary DNA probe sets. Results of the proficiency test with 20 well-characterized test strains sent to 3 participating laboratories for subsequent analysis with the BD GeneOhm™ VanR Assay exactly identified all included *vanA*- and *vanB*-type *Enterococcus* strains (Table 3).

3.2. In vivo study

Parameters of the 2 study institutions are given in Table 2. A total of 1819 rectal swabs from 1026 different patients were included in the study performed at both hospitals. Twenty-nine samples had to be excluded from the analysis: 25 due to questionable quality of specimen (lack of bacterial growth on Columbia blood agar) and 4 due to incomplete documentation. A total of 177 VRE isolates were isolated from 173 specimens. Four samples grew a *vanA*-positive VRE and a *vanB*-positive strain. Of the 177 isolates, 5 were *E. faecalis* (2 *vanA* and 3 *vanB*) and 172 were *E. faecium* (103 *vanA*, 69 *vanB*).

With the use of the solid selective medium, 148 (85%) of the 173 positive samples were detected. Most positive results were seen after 18–28 h of incubation time (122/148, 82%). The positive rate was significantly different in both study institutions after 18–28 h of incubation time, with institution 2 having the higher sensitivity (not shown). However, after 42–52 h of incubation the difference in the recovery rate was no longer significant, but institution 2 performed somewhat better with a higher rate of true-negative results compared to all negative samples. Two hundred twenty-two plates showed growths of black colonies that could not be confirmed as VRE. With the use of the selective broths, 167 (96%) of the 174 positive samples were detected. The bacterial growth in broth was mostly detected after 42–52 h of incubation since only 67 (40%) of the 167 positive results were seen after 18–28 h of incubation time. False-positive results occurred in 403 broths. Recovery rates from broth were not significantly different between the institutions, but institution 2 had a significantly higher rate of true-negative results independently of the incubation time.

Of the total of 1790 tests that were performed with the BD GeneOhm™ VanR Assay, 13 tests (0.7%) gave a result of “unresolved”, indicating inhibition of the PCR reaction. After the test was repeated from the frozen lysed sample, 9 samples gave unambiguous results, 4 samples — of which 1 grew *vanB*-positive VRE — were still inhibited. Thus, 1786 samples were further analyzed. With the use of the BD GeneOhm™ VanR Assay, 88 of the 102 *vanA*-positive specimens, 62 of the 67 *vanB*-positive specimens, 3 of the 4 *vanA*- and *vanB*-positive specimens, and 1403 of the 1613 negative specimens were correctly identified (Table 4). Consequently, the overall concordance between BD GeneOhm™ VanR Assay and culture was 87%. The sensitivity of the test was over 90% independent of the study institution or the kind of *van* gene that was detected (Table 5). However, the specificity of the detection of the *vanB* gene was significantly lower than the specificity of the detection of the *vanA* gene, compared to a culture reference method for the detection of VRE. Depending on the prevalence of the *vanB* gene, the positive predictive value of a positive test result (*vanA* or *vanB*) was significantly lower for institution 1 than for institution 2. The positive predictive value improves significantly if only the *vanA* detection is considered (Table 5).

The BD GeneOhm™ VanR Assay and the reference method produced divergent results in 230 samples (12.9%). The divergent results were confirmed in 198 samples by discrepancy analysis and were resolved in 23 samples. Repeating the BD GeneOhm™ VanR Assay using the original lysate led to results that were concordant with the culture results in 20 samples and in 3 samples after recultivation of the preparation buffer confirming the BD GeneOhm™ VanR Assay result. Corrected for the resolved samples, the sensitivity of the test was 93.4% (95% CI 88.7–96.6) and the specificity was 88.2 (95% CI 86.5–89.7).

A total of 367 patients were tested more than once (maximum 14 times). Of these, 290 patients (79%) had concordant results for all their tests, whereas in 77 patients the test results changed between single tests. Thirty-nine patients had repeatedly negative culture results but intermittently positive BD GeneOhm™ VanR Assay results. In 38 of the patients, the results of culture had changed. In 29 of these patients, BD GeneOhm™ VanR Assay and culture yielded corresponding results. The 9 repeatedly tested patients whose culture results converted to negative/positive and whose PCR and culture results were discordant showed the following patterns: 4 patients were more often *vanA* positive by culture than by BD GeneOhm™ VanR Assay, 2 patients were more often *vanB* positive by BD GeneOhm™ VanR Assay than by culture, 1 patient remained negative by PCR although he became *vanB* culture positive, and 2 patients who became *vanA* culture positive were intermittently tested false positive for *vanB* by BD GeneOhm™ VanR Assay. For 20 patient samples, the BD GeneOhm™ VanR Assay yielded repeatedly false-positive *vanB* results that were not confirmed by culture for VRE (2 to 6 false-positive results).

4. Discussion

The in vitro results showed that the BD GeneOhm™ VanR Assay was capable of identifying all enterococcal reference and test strains investigated in this study that contained *vanA* and *vanB* genes prevalent in different enterococcal species (*E. faecalis, E. faecium, E. mundtii, E. durans, E. gallinarum*). All of the strains with *vanC* to *vanG* genotypes were correctly identified as negative by the assay. Since genotypes *vanE* to *vanG* (and most probably the previously identified *vanL* and *vanM*, too; [Boyd et al., 2008] and [Xu et al., 2010]) still remain rare among clinical enterococcal isolates encountered in hospitals worldwide (Werner et al., 2008), this does not complicate substantially the performance and predictive value of the present assay in detecting and identifying VRE colonizers. Quite contrarily, the prevalence of *vanB*, *vanD*, *vanE*, and *vanG* genes among nonenterococcal, intestinal bacteria may thwart the good performance of a putative assay capable of detecting all acquired *van* genotypes ([Domingo et al., 2005], [Domingo et al., 2007], [Graham et al., 2008], [Launay et al., 2006] and [Tsvetkova et al., 2010]). Performance of the assay in identifying a set of 20 reference and test strains (*vanA*/*vanB* positive and *van* negative) performed at 3 laboratories showed 100% sensitivity and specificity.

The BD GeneOhm™ VanR screening assay was applied at 2 different laboratories of 2 different university hospitals with different VRE prevalence rates among patients. The differences in the performance with the different culture media between the 2 study institutions may be explained by a higher volume of specimens in institution 2 that had a significantly higher VRE rate. However, the positive rates of the tests were not significantly different at the end of the incubation period. Thus it

seems to be warranted to combine the culture data of both institutions as a gold standard for further analysis of the BD GeneOhm™ VanR Assay.

Sensitivity and specificity of the BD GeneOhm™ VanR Assay were concordant within both study institutions. Compared with results of a previous study using the BD GeneOhm™ VanR Assay, the sensitivity was somewhat lower in our study (Stamper et al., 2007). We used a selective broth with a lower concentration of vancomycin (4 µg/mL compared to 8 µg/mL) and the amount used for inoculation of the broth was higher (500 µL compared to 300 µL) suggesting a higher enterococcal yield in our study. On the other hand, the swabs were used to inoculate 2 solid media before the BD GeneOhm™ VanR Assay was performed which may have decreased the sensitivity of the test.

The specificity of the results in our study was concordant with a previous study (Stamper et al., 2007). In another study, Usacheva et al. (2010) compared the results of the BD GeneOhm™ VanR Assay with phenotypical tests only. Therefore their results may be biased by VRE with low MICs and are not comparable to our results.

The low specificity of the detection of the *vanB* gene by various assays has been explained by the presence of Gram-positive, anaerobic bacteria, for example, *Clostridium* spp., *Streptococcus* spp., or *Ruminococcus* spp., that have acquired *vanB* ([Ballard et al., 2005a], [Domingo et al., 2005], [Mevius et al., 1998], [Poyart et al., 1997] and [Stinear et al., 2001]). Since a high percentage of the bacterial community of the bowel has not been cultivated yet, the identification of other vancomycin-resistant microorganisms remains a possibility (Ballard et al., 2005b). In this context, it seems most interesting that the rate of false-positive results was very similar in different populations tested with the same assay, i.e., in the US (Baltimore, MD) and in the 2 different hospitals in southern Germany here, although the prevalence of *vanB*-positive enterococci was somehow different, i.e., 0.6% in Baltimore (Stamper et al., 2007), 2.6% at institution 1, and 4.9% at institution 2 in Germany. Using other real-time–based commercial assays revealed similar results of false-positive *vanB* test results; however, comparator tests were not identical and thus the exact number of these test results is not comparable among each other and with our results ([Bourdon et al., 2010], [Sloan et al., 2004] and [Young et al., 2007]). Nevertheless, one could speculate that the presence of *vanB* containing organisms in the bowel is independent of the population tested and that *vanB* enterococci add to those *vanB*-positive microorganisms already existing in the intestinal tract but occurring largely independently from them. Using animal models, it has been shown that *vanB* genes that are prevalent in anaerobic gut flora can be transferred to enterococci under clinical selective conditions and it has been proposed to include direct *vanB* detection in feces in programs for the control of vancomycin resistance (Launay et al., 2006).

The BD GeneOhm™ VanR Assay was performed in a population of high-risk patients and it can be assumed that many of them had been treated with antibiotics which might increase the selective pressure. Because the antibiotic therapy of the patients has not been documented for the present study, the pattern of antibiotic usage cannot be compared.

A shortcoming of our study may be that the culture method was based on selective media only. We decided to use selective media, because usage of nonselective media would have increased the risk of overgrowth of resistant enterococci by the susceptible flora. But especially *vanB*-positive enterococci may have low MICs to vancomycin which can be lower to what could be detected with the selective broth used in this study (vancomycin 4 µg/mL); for instance, between 5 and 38% of *vanB* positive enterococci sent to the German Focal Laboratory for Enterococci have revealed MICs for vancomycin below or equal to 4 µg/mL (Werner et al., 2007a). Therefore, we may have missed *vanB* strains by culture and this may have resulted in underestimation of the specificity of the *vanB* part of the BD GeneOhm™ VanR Assay.

It is important to note, that at institution 2 the distribution of VRE in clinical samples in which all enterococcal isolates are tested by Vitek 2 (50% *vanA* and 50% *vanB*) was quite similar to the distribution seen in screening samples that were processed by conventional culture (52% *vanA*, 48% *vanB*). This comparison again emphasizes the direct link between VRE colonization and infection (“colonization pressure”; Bonten et al., 1998) and the important role of early recognition, wide screening and the implementation of infection control measures to reduce the burden of VRE infections. Compared to conventional culture the BD GeneOhm™ VanR Assay had twice as many

vanB results than *vanA* results (29% *vanA*, 71% *vanB*) which, in the light of the results just mentioned, indeed suggests a *vanB* reservoir in non-enterococcal, intestinal colonizers.

In conclusion, the BD GeneOhm™ VanR Assay displayed reproducible results in different laboratories and patient populations. It had an excellent negative predictive value and a very good positive predictive value for the detection of *vanA*. However, *vanB*-positive results require confirmation by culture to verify the presence of VRE as is recommended by the manufacturer. Using a combined method of the BD GeneOhm™ VanR Assay followed by a culture-based method in specimens with positive results for *vanB* ("presumptive positives") would only have allowed completing the diagnosis in nearly 85% of specimens on the day of testing. Although this was not the focus of our study, it can be assumed that this approach is cost-effective in institutions where preemptive isolation is part of the infection control program.

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Tables

Table 1. Characteristics of reference and test strains included for the performance testing of the BD GeneOhm™ VanR Assay

Strain	No.[a]	Synonym name	Genotype	Species	Strain characteristics (van genotype, year, MLST type)	MIC of vancomycin (mg/L)	BD GeneOhm™ VanR Assay result	Reference
BM4147	1		*vanA*	*faecium*	Reference for *vanA*	512	POS	Depardieu et al., 2004
V583	1		*vanB*	*faecalis*	Reference for *vanB-1 subtype*	16	Presumptive POS	Paulsen et al., 2003
BM4524	1	UAA 1514	*vanB*	*faecium*	Reference for *vanB-2 subtype*	256	Presumptive POS	Depardieu et al., 2004
VRE45	1	UAA 1842	*vanB*	*faecalis*	Reference for *vanB-3 subtype*	512	Presumptive POS	Depardieu et al., 2004
BA4174	1	UW701	*vanC-1*	*gallinarum*	Reference for *vanC-1*	8	NEG	Devriese et al., 1993
CCM439	1	UW702	*vanC-2*	*casseliflavus*	Reference for *vanC-2*	4	NEG	Devriese et al., 1993
BM4339	1	UAA 768	*vanD*	*faecium*	Reference for *vanD*	256	NEG	Depardieu et al., 2004
BM4539	1	UAA 1562	*vanD*	*faecalis*	Reference for *vanD*	16	NEG	Depardieu et al., 2004
BM4405	1	UAA 942	*vanE*	*faecalis*	Reference for *vanE*	64	NEG	Depardieu et al., 2004
BM4518	1	UAA 1460	*vanG*	*faecalis*	Reference for *vanG*	16	NEG	Depardieu et al., 2004
vanA-type test strains	15		*vanA*	*faecium* (10) *faecalis* (5)	(Outbreak) VRE from France, Germany, Austria; 1990–2005; different MLST types (ST17, -18, -25, -117, -145, -192)[b]	8–1024	POS	Abele-Horn et al., 2006; Klare et al., 2003; Mellmann et al., 2000; Werner et al., 1997; this study
vanB-type test strains	15		*vanB*	*faecium* (10) *faecalis* (3) *mundtii* (1) *durans* (1)	(Outbreak) VRE from Germany and South Africa; 1995–2006; ST17 and ND	4–512	Presumptive POS	Werner et al., 2007b; this study
UW4041	1		*vanC-1*; *vanA*[c]	*gallinarum*	Clinical isolate from Germany, 2003	128	POS	This study
vanC-type test strains	6		*vanC-1*	*gallinarum*	Clinical isolates from Germany; 2006–2007	2–8	NEG	This study
vanC2-type test strains	4		*vanC-2*	*casseliflavus*	Clinical isolates from Germany; 2004–2005	8	NEG	This study

ND = Not determined.
a Number of investigated strains.
b Sequence type based on MLST (shown here only for E. faecium isolates).

c Additional vanA gene cluster in vanC1-type E. gallinarum.

Table 2. Characteristics of the 2 study institutions (university hospitals)

	Institution 1	Institution 2
Kind of hospital	University hospital	University hospital
No. of beds	1480	1600
Screening program	High-risk patients	High-risk patients and all patients from high-risk units
Preemptive isolation	No	Contact patients of known VRE carrier
No. and kind of hospital units that sent screening specimens	3 ICUs, 17 standard care units	11 ICUs, 31 standard care units
Time of study	10/2007–04/2008	9/2007–01/2008
No. of specimens	755 (14 from ICU)	1064 (981 from ICU)
No. of patients	375	676
No. of VRE-positive specimens	43	130
VRE-positive patients	19	94
VRE prevalence among screened patients	5.1%	13.9%

Table 3. Results of the proficiency test with a set of 20 strains tested in a blinded trial at 3 independent laboratories

Strain	Strain characteristics (year, MLST type)	Species	*vanA*	*vanB*	BD GeneOhm™ VanR Assay result			Reference
					Partner 1	Partner 2	Partner 3	
UW1833	Outbreak VRE from Germany, 1998, ST40[a]	*faecalis*	+	−	POS	POS	POS	This study
UW3447	Clinical VSE from Germany, 2001, ST30	*faecalis*	−	−	NEG	NEG	NEG	This study
UW4969	Clinical VSE from Germany, 2004, ST55	*faecalis*	−	−	NEG	NEG	NEG	This study
UW6490	Clinical VRE from Germany, 2006, ST55	*faecalis*	+	−	POS	POS	POS	This study
UW7156	Clinical VRE from Germany, 2007	*faecium*	−	+	Presumptive POS	Presumptive POS	Presumptive POS	This study
UW7002	Clinical VSE from Germany, 2007, ST6	*faecalis*	−	−	NEG	NEG	NEG	This study
UW7007	Clinical VSE from Germany, 2007, ST4	*faecalis*	−	−	NEG	NEG	NEG	This study
UW7008	Clinical VSE from Germany, 2007, ST55	*faecalis*	−	−	NEG	NEG	NEG	This study
V583	Reference strain for *vanB*	*faecalis*	−	+	Presumptive POS	Presumptive POS	Presumptive POS	Paulsen et al., 2003
JH2-2	Vancomycin-susceptible recipient	*faecalis*	−	−	NEG	NEG	NEG	Dunny and Clewell, 1975
UW6494	Clinical VRE from Germany, 2006, ST78	*faecium*	+	−	POS	POS	POS	This study
UW6595	Clinical VRE from Germany, 2006, ST17	*faecium*	+	−	POS	POS	POS	This study
UW6601	Clinical VRE from Germany, 2006, ST65	*faecium*	+	−	POS	POS	POS	This study
UW6666	Clinical VRE from Germany, 2006, ST18	*faecium*	+	−	POS	POS	POS	This study
UW6716	Clinical VSE from Germany, 2006, ST317	*faecium*	−	−	NEG	NEG	NEG	This study
UW6918	Clinical VRE from Germany, 2006, ST18	*faecium*	−	+	Presumptive POS	Presumptive POS	Presumptive POS	This study
UW6923	Clinical VSE from Germany, 2006, ST202	*faecium*	−	−	NEG	NEG	NEG	This study
UW6984	Clinical VSE from Germany, 2006, ST192	*faecium*	−	−	NEG	NEG	NEG	This study
BM4147	Reference for *vanA*	*faecium*	+	−	POS	POS	POS	Depardieu et al., 2004
64/3	Vancomycin-susceptible recipient	*faecium*	−	−	NEG	NEG	NEG	Werner et al., 1997

Table 4. Comparison of results of BD GeneOhm™ VanR Assay versus culture followed by PCR

		BD GeneOhm™ VanR Assay				Total
		vanA	*vanB*	*vanA* and *vanB*	Negative	
Culture and PCR	*vanA*	88		6	8	102
	vanB		62	1	4	67
	vanA and vanB		1	3		4
	Negative	5	204	1	1403	1613
	Total	93	267	11	1415	1786

Table 5. Performance characteristics of the BD GeneOhm™ VanR Assay compared to culture followed by PCR stratified by study institution

	vanA	*vanB*	VRE
Prevalence [%] with 95% confidence interval (CI_{95})			
Institution 1	3.1 (2.0–4.6)	2.6 (1.7–4.8)	5.7 (4.2–7.7)
Institution 2	7.8 (6.3–7.9)	4.9 (3.7–6.5)	12.6 (10.7–14.8)
Combined	5.9 (4.9–7.7)	4.0 (3.1–5.0)	9.7 (8.4–11.2)
Sensitivity [%] with CI_{95}			
Institution 1	91.3 (70.5–98.5)	95.0 (73,0–99.7)	93.0 (79.9–98.2)
Institution 2	93.8 (85.5–97.7)	94.1 (82.8–98.5)	93.1 (86.9–96.6)
Combined	91.5 (84.1–95.8)	94.4 (85.5–98.2)	93.1 (87.9–96.2)
Specificity [%] with CI_{95}			
Institution 1	99.7 (98.9–99.9)	86.5 (83.8–88.8)	85.9 (83.1–88.4)
Institution 2	99.3 (98.4–99.7)	88.6 (86.4–90.5)	87.8 (85.4–89.8)
Combined	99.6 (99.1–99.8)	87.7 (86.0–98.2)	87.0 (85.2–88.6)
Positive predictive value [%] with CI_{95}			
Institution 1	91.3 (70.5–98.5)	16.1 (10.2–24.2)	28.8 (21.4–36.9)
Institution 2	91.6 (82.8–96.2)	30.0 (23.2–37.8)	52.4 (45.7–58.9)
Combined	93.3 (86.1–97.0)	24.1 (19.3–29.6)	43.4 (38.3–48.6)
Negative predictive value [%] with CI_{95}			
Institution 1	99.7 (98.9–99.9)	99.8 (99.0–100)	99.5 (98.4–99.9)
Institution 2	99.5 (98.7–99.8)	99.6 (98.9–99.9)	98.9 (97.8–99.4)
Combined	99.5 (98.9–99.7)	99.7 (99.3–99.9)	99.1 (98.5–99.5)