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Comparison of the BD GeneOhm VanR Assay to Culture for Identification of Vancomycin-Resistant Enterococci in Rectal and Stool Specimens▽

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Active screening for vancomycin-resistant enterococci (VRE) in rectal and stool specimens has been recommended to limit the spread of antimicrobial resistance within certain high-risk populations. Directly from 502 rectal swabs and stool specimens, we evaluated the diagnostic performance of the BD GeneOhm VanR assay (BD GeneOhm, San Diego, CA), a rapid real-time PCR test that detects the presence of *vanA* and/or *vanB* genes. The VanR assay was compared to culture consisting of both bile-esculin-azide agar with 6 μg/ml vancomycin (BEAV agar) (BD Diagnostics, Sparks, MD) and BEAV broth with 8 μg/ml vancomycin (Hardy Diagnostics, Santa Maria, CA). Enterococci were identified to the species level using standard biochemical tests and a Phoenix automated microbiology system (BD Diagnostics, Sparks, MD). The susceptibility of the enterococci to vancomycin and teicoplanin was determined using an Etest (AB Biodisk, Solna, Sweden). VRE were initially isolated from 147 cultures, and the VanR assay detected 142 of the 147 positive cultures for a sensitivity of 96.6%. The specificity was 87.0% (309/355) largely due to false positives seen with the *vanB* portion of the assay. The sensitivity when testing rectal swabs was 98.3%, and the sensitivity for stool samples was 95.4% ($P = 0.643$). The specificity of rectal swabs was comparable to that of the stool specimens (87.5% and 86.5%, respectively). When used only to detect VanA resistance, the VanR assay was 94.4% (136/144) sensitive and 96.4% (345/358) specific, with positive and negative predictive values of 91.3% and 97.7%, respectively. In summary, the BD GeneOhm VanR assay is a good screening test for VRE in our population of predominantly *vanA*-colonized patients. However, patient samples testing only *vanB* positive should be confirmed by another method for the presence of VRE.

Although not observed in United States hospitals until 1987 (26), vancomycin-resistant enterococci (VRE) are a clinical problem that, as predicted, account for an ever-increasing proportion of the enterococcal infections in United States hospitals (3, 10, 35). A number of mechanisms mediating glycopeptide resistance exist in enterococci, but the *vanA*- and *vanB*-resistant strains are the most common pathogenic VRE found in the United States (7, 31).

The prevalence of VRE can be difficult to discern, as those patients at the highest risk for colonization and infection frequently move between hospital wards, intensive care units (ICUs), and long-term-care facilities. In a study of 60 hospital ICUs, the average VRE prevalence was 10.0%, with a wide range (0% to 59%) depending upon the institution and type of unit surveyed (12). In Baltimore, MD, 10.1% of patients admitted to the medical ICU or surgical ICU with a 5-h stay or longer were colonized (13). Cosgrove has shown that those patients with VRE infections have over twice the risk of dying, will be hospitalized longer, and are more likely to be admitted to an ICU (6). When VRE is the cause of bacteremia, the risk of death dramatically increases (2, 14); several studies show an independent association of vancomycin resistance and mortality (8, 9). Because of the aforementioned morbidity and mortality, infection with VRE significantly increases hospital costs (6).

The length of stay in a health care facility and prior exposure to antibiotics are known risk factors for VRE colonization, but other risk factors identifying VRE carriers are less clear (18). Some authors are proponents of active surveillance for VRE, while others advocate screening only after a baseline threshold has been crossed (30). In any approach, a rapid, accurate tool to identify carriers is a key component (20, 28, 33) of any infection control program to reduce transmission.

The detection of VRE has traditionally relied upon culture, which requires 24 to 72 h for isolation, identification, and susceptibility testing and has no clear advantage over selective media (21, 32). Culture has been the method of choice, but a screening assay that could identify patients colonized with VRE in <24 h would facilitate more rapid implementation of patient isolation precautions. Several nucleic acid amplification tests have been developed and evaluated for the detection of VRE, but in many circumstances the direct tests require complicated extraction and detection regimens (11, 22, 23, 27, 29) or require a culture step, testing either from a selective enrichment broth (1, 12) or isolates recovered from solid media (24, 25). The BD GeneOhm VanR assay (BD GeneOhm, San Diego, CA) is a U.S. Food and Drug Administration-cleared in vitro test for VRE screening directly from perianal or rectal swabs but not from stool samples. The BD GeneOhm

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VanR assay uses a simple processing method, and the assay is performed on a Cepheid SmartCycler (Cepheid, Sunny vale, CA). This platform allows samples to be tested individually or in groups depending on the machine configuration and number of specimens. The results are available in less than 3.4 h from specimen extraction to interpretation. This study compared the performances of the BD GeneOhm VanR assay and culture using selective agar and broth for the detection of VRE from surveillance specimens.

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MATERIALS AND METHODS

Patients and specimens. This industry-sponsored study was performed according to the manufacturer's protocol for FDA submission and was approved by the Johns Hopkins University institutional review board. Eligible participants were those patients who had a stool sample or a rectal/perianal swab submitted to the clinical microbiology laboratory of the Johns Hopkins Hospital for routine VRE screening, were older than 18 years of age, and could consent or had a consignee available to consent for participation. Stool specimens were submitted in sterile containers collected in the patient's room. Alternatively, a single rectal/perianal swab was obtained by nursing staff using the liquid Stuart's BBL CultureSwab collection and transport system (BD Diagnostics, Sparks, MD). On the days of enrollment, eligible patients (n = 412) or their legal representatives were approached and 404 individuals agreed to participate (98%). With the sponsor's approval, up to 25% of participants were allowed to submit a second sample during the course of the study; thus, a total of 502 samples were tested, 252 rectal swabs and 250 stool samples.

Specimen processing. A sterile cotton swab (Medline, Mundelein, IL) was introduced into well-mixed fecal material from a liquid or semisolid stool specimen, and then the stool swab was treated the same as a rectal/perianal swab. Specimen processing and medium inoculation were performed in a biological safety cabinet. The single rectal or stool swab was used to inoculate a bile-esculin-azide agar plate (BEAV agar), with 6 μg of vancomycin (BD Diagnostics, Sparks, MD), and the tip of the swab was broken off into a VanR sample buffer tube containing a Tris-EDTA sample preparation buffer, provided by the manufacturer. After the sample buffer tube was vortexed for 1 min, 300 μl of the solution was inoculated into BEAV VRE broth, with 8 μg of vancomycin (Hardy Diagnostics, Santa Maria, CA), for culture, and 50 μl of the solution was transferred into a VanR lysis tube for further PCR processing.

Culture method. All primary culture plates and broths were incubated in 5 to 10% CO_2 at 35 to 36°C. The sponsor's FDA clinical trial testing algorithm to detect VanA and VanB vancomycin resistance using the selective and differential media for *Enterococcus* species was performed as follows: cultures were reviewed daily before being called negative on the third day of review. Brown to black colonies on BEAV plates were Gram stained, and gram-positive cocci were considered presumptively positive for VRE. Black discoloration or cloudiness in the BEAV broth was considered a positive result. The positive broth was subcultured to a BEAV plate and read in a manner similar to that for the directly inoculated BEAV plate. Bacterial isolates on BEAV plates were isolated on 5% sheep blood agar (SBA) and identified using standard rapid tests and biochemical media for the identification of gram-positive cocci. For *Enterococcus* species-level identification, catalase-negative, L-pyrrolidonyl-β-naphthylamide enzyme-positive, gram-positive cocci from SBA were further examined with biochemicals incorporated into agar slants that were read at 24 h of aerobic incubation at 35 to 36°C. Only motility agar was incubated at room temperature. The following tests were used to differentiate *Enterococcus* species: motility; pigment production; hydrolysis of the carbohydrate esculin; growth in the presence of 6.5% sodium chloride; acidification of the sugars (1%) raffinose, arabinose, sucrose, lactose, mannitol, sorbitol, and sorbose; acidification of methyl-α-*d*-glucopyranoside; and detection of the enzyme arginine decarboxylase (19). A Phoenix automated rapid identification system (BD Diagnostics, Sparks, MD), which uses fluorogenic and chromogenic biochemical substrates, was used to assist with species identification of any isolate not readily identified by the conventional rapid tests and biochemicals (4).

Susceptibility testing was performed on confirmed enterococcal isolates using vancomycin (0.016 to 256 μg/ml) and teicoplanin (0.016 to 256 μg/ml) Etest strips (AB Biodisk, Solna, Sweden). The determination of the MICs and the interpretation of vancomycin resistance (MIC ≥ 32 μg/ml) were done according to Clinical and Laboratory Standards Institute (CLSI) guidelines (5). For the interpretation of the teicoplanin results, we combined the intermediate and resistant MICs according to the sponsor's algorithm for the assignment of isolates as having VanA (MIC, ≥16 μg/ml) or VanB (MIC, <16 μg/ml) phenotypes.

VanR assay. After inoculation with the swab as mentioned previously, 50 μl of sample buffer was transferred into a lysis tube containing glass beads which uses physical action to lyse cells. The lysis tube was vortexed for 5 min at high speed and received a quick spin in the centrifuge to bring the contents to the bottom of the tube before incubation at 95°C in a dry block for 2 min. The sample was kept at 3 to 5°C until testing was preformed. A SmartCycler tube to which 25 μl of the reconstituted master mix was added was used for each sample. After the addition of the master mix, 3 μl of the sample from the prepared sample lysis tube was added to a correspondingly labeled tube. The SmartCycler tubes were always closed after each sample was added. The PCR-positive control for each run was a reconstituted DNA control provided in the BD GeneOhm VanR assay kit. Uninoculated sample buffer was added to the last sample tube for use as a negative control. All tubes were kept at 3 to 5°C while being prepared and inoculated. The processed lysis tubes were stored at −20°C after the PCR was initiated.

Prior to being placed in the SmartCycler I-CORE module, the reaction tubes were centrifuged briefly to bring the solution (master mix and sample) into the reaction chamber. The run was started according to the manufacturer's software and amplification protocol for the BD GeneOhm VanR assay, using the SmartCycler software. The BD GeneOhm VanR assay is a real-time PCR assay containing proprietary primers that amplify specific targets of the *vanA* and/or *vanB* genes. Amplified targets are detected with unique fluorescent hybridization probes (molecular beacons). The assay also contains a well-documented internal control (IC) (11, 16). The BD GeneOhm VanR assay uses three channels simultaneously, allowing the SmartCycler analyzer to qualitatively report the detection of *vanA* and *vanB* genes and IC amplification. The SmartCycler software interprets the fluorescence growth curve of each reaction mixture and the IC using a decisional algorithm set according to the programmed BD GeneOhm VanR assay parameters. The assay results were interpreted as follows: "negative," no *vanA* or *vanB* DNA was detected; "positive," *vanA* and/or *vanB* DNA was detected; "unresolved," the IC was inhibited or there was reagent failure; "invalid assay run," the PCR control (positive or negative) failed; and "not determined," there was an I-CORE module malfunction. Samples that gave equivocal results were retested. If the IC failed to amplify upon retesting, a 1:20 dilution of sample to sample buffer was made from the original frozen sample buffer tube and then the PCR assay was repeated from the original frozen lysate and the diluted lysate (1:20 dilution of sample buffer).

Quality control. All bacterial culture methods, susceptibility testing, and biochemical reagent measurements were performed according to standard laboratory procedures. In addition to the previously mentioned PCR control for each run, on each day of BD GeneOhm VanR assay processing, a positive control, either a *vanA*-positive *Enterococcus faecium* isolate (characterized clinical isolate) or a *vanB*-positive *Enterococcus faecalis* strain (ATCC 51299), was included to serve as an external extraction control. Regularly, a negative-extraction control (a characterized vancomycin-sensitive *E. faecium* isolate or *E. faecalis* strain ATCC 29212) was added to test for potential contamination.

Discrepant analysis. Culture and PCR results were reviewed by a microbiologist independent of the one who conducted the initial testing. Samples in which the culture result and the BD GeneOhm VanR assay result differed were tested again in duplicate using the original frozen lysate according to the clinical trial study protocol. The test results from the first BD GeneOhm VanR assay PCR were used for the primary analysis of the data. Further adjudication was attempted with culture if the rerun duplicate real-time PCR did not resolve the discrepant result: for samples in which the PCR was negative but the culture was VRE positive, the VRE enrichment broth and any corresponding isolates were tested by the BD GeneOhm VanR assay. BD GeneOhm VanR assay-positive *vanA* or *vanB* samples which were culture negative for *Enterococcus faecalis* or *Enterococcus faecium* (the clinical trial endpoint) were examined again by subbing the enrichment broth to SBA and testing the VRE enrichment broth using the BD GeneOhm VanR assay. Isolates were identified as previously described.

Data analysis. Culture-positive results of the BD GeneOhm VanR clinical trial were determined as negative or positive for VRE species (vancomycin MIC, ≥32 μg/ml). The isolates from positive cultures were further categorized by glycopeptide resistance as phenotypically consistent with VanA or VanB using the following teicoplanin susceptibility results: VanA phenotype, teicoplanin MIC of ≥16 μg/ml (intermediate interpretation by CLSI), and VanB phenotype, teicoplanin MIC of <16 μg/ml. The PCR results were determined according to the manufacturer's specifications for the BD GeneOhm VanR assay with the

TABLE 1. Enterococcus isolates recovered from initial cultures

Organism	No. of isolates with indicated vancomycin susceptibility				Total no. of isolates
	Rectal swab		Stool		
	<32 μg/ml	≥32 μg/ml	<32 μg/ml	≥32 μg/ml	
E. casseliflavus	7	0	4	0	11
E. gallinarum	12	0	14	1	27
E. durans	0	0	0	1	1
E. faecium	0	53	3	82	138
E. malodoratus	0	0	1	0	1
E. raffinosus	3	2	0	1	6
E. faecalis	0	9	2	4	15
Total	22	64	24	89	199

TABLE 2. Initial results with VanR assay compared to culture

Culture result[a]	No. of isolates positive by VanR PCR for:			No. of isolates negative by VanR PCR	Total
	vanA	*vanB*	*vanAB*		
VanA phenotype	110	3	26	5	144
VanB phenotype	0	3	0	0	3
Negative	11	33	2	309	355
Total	121	39	28	314	502

[a] VanA phenotype, teicoplanin MIC of ≥16 μg/ml; VanB phenotype, teicoplanin MIC of <16 μg/ml.

SmartCycler software as negative, either *vanA* positive or *vanB* positive, or positive for both *vanA* and *vanB* (*vanAB*).

Descriptive statistics were determined for the rectal swabs, stools, and all samples combined. An additional analysis was done with the adjudicated discrepant results included. The comparison tests of means, Fisher's exact test, and Pearson's chi-square analysis were used when appropriate to measure association and to determine statistical significance with Stata 7.0 (Stata Corporation, TX).

RESULTS

Culture. A total of 502 samples were evaluated (252 rectal swabs and 250 stool specimens). Three hundred fifty-five samples were negative for any VRE by culture and 147 samples were VRE positive, for an overall initial culture positivity rate of 29.3%. Several samples contained multiple isolates of vancomycin-susceptible and -resistant enterococci. Table 1 lists the enterococci recovered by culture, stratified by vancomycin susceptibility. A total of 135 vancomycin-resistant *E. faecium* and 13 vancomycin-resistant *E. faecalis* isolates were recovered. All of the vancomycin-resistant *E. faecium* isolates initially recovered were very resistant to vancomycin (MIC, ≥64.0 μg/ml). Nine samples grew vancomycin-resistant *E. faecalis* in pure culture, and four cultures were mixed, with both resistant *E. faecium* and resistant *E. faecalis*. Only three of the *E. faecium* isolates exhibited a typical VanB phenotypic resistance pattern for teicoplanin (MIC, <16 μg/ml) as defined by the study protocol, and all three were PCR positive for *vanB* alone. There was a difference in the prevalence of VRE seen between rectal swabs (60/252; 23.8%) and stool samples (87/250; 34.8%).

From the 147 VRE-positive cultures, 153 VRE isolates were recovered, five of which were vancomycin-resistant species other than *E. faecium* or *E. faecalis* (three *E. raffinosus*, one *E. durans*, and one *E. gallinarum*). Forty-six additional enterococci grew on the BEAV agar or were recovered from BEAV broth but were not VRE (vancomycin MIC, <32 μg/ml) (Table 1).

PCR. An overall comparison of the results for the VanR assay and the culture results is presented in Table 2. One hundred eighty-eight samples tested positive for *vanA* and/or *vanB*. There were two rectal swab samples that were culture negative but were classified as "unresolved" by the BD GeneOhm VanR assay software due to inhibition of the PCR. However, both resolved—one on repeat testing and one after a 1:20 dilution of the original sample buffer. As these samples were easily resolved with no extraordinary procedures, both were included in the initial analysis. Three hundred nine samples were negative by both culture and the VanR assay, and 142 were positive by both culture and the BD GeneOhm VanR assay, for an overall agreement of 89.8%.

Six samples tested only *vanB* positive by the BD GeneOhm VanR assay, but three of the isolates possessed VanA phenotypes, with MICs to vancomycin of ≥256 μg/ml. Two of these organisms had MICs to teicoplanin of ≥256 μg/ml, and one had a MIC of 32 μg/ml. Interestingly, these isolates were *E. raffinosus* ($n = 2$) and *E. faecium* ($n = 1$). The isolates recovered from the 26 samples testing *vanAB* positive by the BD GeneOhm VanR assay were all phenotypically VanA.

The sensitivity, specificity, positive predictive value, and negative predictive value for the BD GeneOhm VanR assay compared to those of culture, before resolution of the discrepant samples and stratified by specimen type, are presented in Table 3. The VanR assay had an overall sensitivity of 96.6% and a specificity of 87.0%. The positive predictive value and negative predictive value in our population were 75.5% and 98.4%, respectively. The sensitivity of the BD GeneOhm VanR assay was higher using rectal swabs (98.3%) than using stool samples (95.4%), but this difference was not statistically significant ($P = 0.643$). The specificity was very similar for the rectal swabs and the stool samples, 87.5% and 86.5%, respectively ($P = 0.874$). Upon analyzing the data for *vanA*-mediated resistance alone, the most common type of VRE resistance found in our isolates, we found that the sensitivity of the assay was 94.4% and the specificity for *vanA* was 96.4%. The *vanB* portion of the assay was problematic. While the three isolates that were phenotypically VanB were all detected by the PCR assay, the VanR assay reported positive results for *vanB* for 33 culture-negative samples (see Table 2 and discussions below).

Discrepant samples. Fifty-one specimens gave discrepant results: 5 were culture positive, VanR negative; 13 were culture negative, *vanA* or *vanAB* positive; and 33 were culture negative, *vanB* positive (Table 2).

(i) Culture-positive, VanR-negative samples. Of the five VRE culture-positive, VanR-negative samples, one isolate was *E. gallinarum*, growing in the broth and on the plate, and the other four were *E. faecium* strains. Three of the *E. faecium* isolates grew in BEAV broth and on BEAV agar and one grew only on the BEAV agar plate. The VanR assay was repeated using the original lysates, and the results were negative upon

TABLE 3. Performance characteristics of the VanR assay compared to culture stratified by specimen type

Sample type(s)	Sensitivity			Specificity			Positive predictive value			Negative predictive value		
	No.[b]	%	95% CI	No.	%	95% CI	No.	%	95% CI	No.	%	95% CI
Rectal swabs[a]	59/60	98.3	94.0–100	168/192	87.5	82.8–92.2	59/83	71.1	61.3–80.8	168/169	99.4	97.8–100
Stool samples[a]	83/87	95.4	91.0–99.8	141/163	86.5	81.3–91.8	83/105	79.0	71.3–86.8	141/145	97.2	94.6–99.9
Both sample types combined[a]	142/147	96.6	93.7–99.5	309/355	87.0	83.6–90.5	142/188	75.5	69.4–81.7	309/314	98.4	97.0–99.8
Both sample types, PCR *vanA*/*vanAB*	136/144	94.4	90.7–98.2	345/358	96.4	94.4–98.3	136/149	91.3	89.3–97.3	345/353	97.7	96.2–99.3

[a] VanR overall: *vanA*/*vanB*/*vanAB* PCR.
[b] Number of positive samples/total number.

retesting. The four *E. faecium* isolates themselves tested *vanA* positive. The *E. gallinarum* was not tested for non-*vanA*- or non-*vanB*-mediated glycopeptide resistance.

(ii) Culture-negative, *vanA* or *vanAB*-positive samples. Initially, 11 samples were *vanA* positive and 2 were *vanAB* positive with the BD GeneOhm VanR assay but were culture negative. These 13 samples were retested using the VanR assay, and 11 of the lysis buffers gave the same result as the initial testing at least once with duplicate retesting done by the BD GeneOhm VanR assay. The BD GeneOhm VanR assay PCR was done on BEAV VRE broth, and seven broth cultures were positive for *vanA* or *vanAB*. These seven broths were recultured, and vancomycin-resistant *E. faecium* was isolated from the broth of three of the cultures on the second attempt. These three samples that grew *E. faecium* were considered positive only for the secondary analysis.

Three of the discrepant cultures grew *E. raffinosus*, and the isolates tested *vanA* positive with the BD GeneOhm VanR assay. The phenotypes of the three *E. raffinosus* were susceptible—their vancomycin MICs ranged from 2 to 4 μg/ml and their teicoplanin MICs were equal to 4 μg/ml. As these were positive for the *vanA* genotype but were not phenotypically VRE, they were not considered positive in the initial or revised analysis.

(iii) Culture-negative, vanB-positive samples. There were 33 samples that were culture negative but *vanB* positive originally from the lysis buffer, and upon repeat PCR testing, 28 samples were again PCR positive for *vanB*, whereas 5 did not have repeat positive results by the *vanB* PCR. Twenty-seven of the 28 original BEAV broths in which the lysis buffer tested *vanB* positive were cultured again aerobically and anaerobically in a second attempt to recover isolates containing *vanB*. Seven of the repeat cultures grew microorganisms, but only one isolate from the culture tested positive by the *vanB* PCR. The isolate was *E. faecium*, and in the secondary analysis, this was counted as a positive.

When the results from rectal swabs and stool samples were combined, the overall sensitivity was 95.4%. The specificity, positive predictive value, and negative predictive value were improved with the inclusion of the resolved discrepant results, to 88.0%, 77.7%, and 98.4%, respectively.

DISCUSSION

The BD GeneOhm VanR assay performed equally well directly from rectal swabs and from stool samples. The performance of the BD GeneOhm VanR assay was comparable to that of standard VRE culture techniques at our institution, with an overall sensitivity of 95 to 97% (initial and secondary analysis). The *E. faecium* isolates, from four of the five false-negative samples by the VanR assay, were *vanA* positive when retested by the BD GeneOhm VanR assay. These four results are considered false negatives by the BD GeneOhm VanR assay. One explanation for these results is that the concentration of the *E. faecium* in those samples could have been near the limit of detection of the assay. According to the manufacturer, the limit of detection of the VanR assay is 10 genome copies per reaction for both *vanA* and *vanB* VRE, which equates to about 2 CFU/reaction. Taking into account a dilution factor due to specimen processing, this translates into approximately 1,600 CFU/swab.

The VanR assay was only 87% specific upon initial testing, improving only slightly to 88% after resolution in the secondary analysis. No typical VRE was isolated from primary culture for 13 false-positive *vanA* or *vanAB* samples. Repeat testing from the original lysates yielded VanR-positive results for 11, and these 11 were further examined. Reculture of the BEAV broths resolved three of these discrepant results with the isolation of *E. faecium*. On the secondary analysis, these three were now considered as culture positives. During initial testing, 33 samples were culture negative but *vanB* positive using the BD GeneOhm VanR assay, and upon further examination, only one of these was found to contain a *vanB*-positive isolate (*E. faecium*). Overall, the poor specificity was attributed primarily to false-positive results with the *vanB* portion of the assay (33/39; 85%). This has been reported by others using various assays for *vanB* detection and is most likely due to the presence of gram-positive anaerobic bacteria, namely, *Clostridium* sp. and *Eggerthella lenta*, that have also acquired *vanB* (1, 11). This may present a problem for laboratories that see predominantly *vanB*-containing VRE in their institutions. In such environments, VanR-positive results would require culture confirmation. In a hospital that has predominantly *vanA*-containing VRE, this assay has excellent positive predictive value and would not require culture confirmation.

As expected, the majority of the VRE were *E. faecalis* or *E. faecium.* Five isolates (three *E. raffinosus*, one *E. durans*, and one *E. gallinarum*) exhibited a VanA resistance phenotype. One of these *E. raffinosus* isolates and the *E. durans* isolate contained a *vanA* genotype, and the other two *E. raffinosus* isolates were *vanB* positive. Three cultures grew *E. raffinosus* isolates that tested phenotypically vancomycin susceptible (vancomycin MIC, ≤4 μg/ml) from the BEAV agar and were considered false-positive results by the VanR assay even

though the isolates exhibited a *vanA* genotype by the VanR assay. *E. raffinosus* and *E. durans* containing *vanA* or *vanB* have been mentioned in literature reviews, but very rarely have enterococcal species other than *E. faecalis* or *E. faecium* containing *vanA* or *vanB* been reported (15, 34). These isolates necessitate further study. It is possible that current phenotypic screening methods fail to consistently differentiate *E. raffinosus* from other *Enterococcus* species. The epidemiological significance of *vanA* or *vanB* VRE which are not *E. faecalis* or *E. faecium* strains requires elucidation. Presumably such species can serve as reservoirs for the transmission of vancomycin resistance.

The BD GeneOhm assay is considerably faster than culture even when set in a convenient laboratory work schedule that restricts PCR testing to once per day (batch mode) with no weekend PCR testing. During the trial, the median time to completion of culture was 74.2 h and the median time to results for the PCR assay was 27 h. The difference in time to results between the BD GeneOhm VanR assay and culture was statistically significant ($P = 0.000$). The assay itself requires no more than 3.4 h from the time of arrival of the specimen at the laboratory until final results are available. PCR inhibition directly from stool samples (17) has been a continual problem, and those developing VRE diagnostic tests have encountered this difficulty when testing clinical samples using a variety of extraction methods (18). Most nucleic acid amplification assays have not adequately addressed the inhibition rate (23, 24, 27), and the efficiency of these assays postimplementation has not been well described. Similar to the results of Domingo et al. using the same extraction method with rectal swabs (11), we encountered surprisingly little sample inhibition with rectal swabs and stool samples. The two inhibited samples were easily resolved with repeat testing. The assay performance was consistent during the 17 weeks of use.

Using the VanR assay as a screening method, an institution could rapidly identify VRE carriers, given the high overall sensitivity and negative predictive values. A diagnostic tool to more quickly determine patients who are colonized would facilitate any multifaceted infection control policy. When used to identify VRE in areas where *vanA*-containing isolates are most prevalent, *vanA*-positive or *vanAB*-positive results would not require culture confirmation. In contrast, the high number of false positives for *vanB* will necessitate culture confirmation of those results. Laboratories will need to weigh the convenience of rapid negative results with the requirement for additional testing and to assess whether such confirmatory testing leads to delayed reporting.

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