Comparison of PCR and Culture for Screening of Vancomycin-Resistant Enterococci: Highly Disparate Results for vanA and vanB

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We compared PCR to conventional culture for the detection of vancomycin-resistant enterococci (VRE) in 30,835 rectal samples over a 3-year period. The positive and negative predictive values of vanB PCR were 1.42% and 99.9%, respectively. A positive vanB result by PCR is poorly predictive and necessitates culture for differentiation of VRE-positive and -negative individuals.

Vancomycin-resistant enterococci (VRE) are multidrug-resistant colonizers of the gastrointestinal tract and have emerged as an important cause of nosocomial infections. Glycopeptide resistance is mediated by six different vancomycin genes, of which vanB remains the most clinically relevant of the Van genes as they are associated with transposons and may theoretically mediate horizontal transfer of vancomycin resistance to other organisms (5). Phenotypically, the vanA gene mediates high-level resistance to vancomycin and teicoplanin while the vanB gene confers low- to moderate-level resistance to vancomycin only. There are three subtypes of vanB: vanB1, vanB2, and vanB3. In addition to enterococci, the vanB genes have been described in a Streptococcus mitis strain isolated from blood (8) and a Streptococcus bovis isolate (11), as well as Eggerthella lenta, a Ruminococcus lactaris-like organism, and several Clostridium species isolated from human feces (1, 2, 4). The presence of vanB-containing organisms other than VRE in stool would decrease the specificity of VRE PCR testing (3, 7, 9, 13, 14). In our study, we compared PCR to simultaneous selective culture for screening rectal swabs for VRE.

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From October 2004 to November 2007, surveillance was performed during periods of VRE outbreaks by both PCR and conventional culture. Rectal swabs were obtained from the following high-risk populations: all admissions through the Emergency Department, including patient transfers from other institutions; inpatients from hospital units with VRE-positive patients; and inpatients with diarrhea whose stools were received for Clostridium difficile toxin testing in the microbiology laboratory. Finally, periodic screening of dialysis patients was performed at intervals determined by the Infection Prevention and Control Unit.

Rectal swabs were soaked in 0.2 ml of saline, and DNA was extracted using the MagNA Pure compact nucleic acid isolation kit 1 on a MagNA Pure compact DNA extractor (Roche Applied Science). The Roche LightCycler VRE detection kit in a Roche LightCycler 2.0 VRE detection platform (Roche Diagnostics GmbH, Germany) was used to identify vanA and vanB (vanB1 or vanB2 or vanB3). Concurrently, 100 μl of the saline from the rectal swab was plated onto Enterococcosel/vanco agar containing 6 μg/ml of vancomycin (Enterococcosel/vanco) and incubated for 48 h at 35°C under aerobic conditions. Black (esculin-positive) colonies were then subcultured onto a blood agar plate for purity. Enterococcosel/vanco isolates were identified with a compatible Gram stain, negative catalase reaction, positive pyrrolidonyl arylamidase test, and growth in 6.5% sodium chloride. Testing for susceptibility to vancomycin and teicoplanin was determined by Etest (AB Biodisk, Sweden) according to the manufacturer’s procedure. The organisms were presumptively identified as VRE if the vancomycin MIC was equal to or greater than 8 μg/ml or if the isolate grew repeatedly on Enterococcosel/vanco agar, regardless of the vancomycin MIC. All suspected VRE were sent to the Quebec public health laboratory for confirmation of genus and species with 16S rRNA sequencing and MIC testing of vancomycin, ampicillin, quinupristin-dalfopristin, and teicoplanin by broth microdilution testing. In addition, vanA gene PCR and pulsed-field gel electrophoresis were performed for epidemiologic typing.

A total of 30,835 rectal specimens were obtained from 12,983 patients (Table 1). Real-time PCR and conventional culture were performed simultaneously for 30,367 specimens. Four hundred sixty-eight specimens did not undergo PCR testing for various reasons including mislabeling, loss of specimen, and multiple concurrent specimens sent. The overall prevalence of VRE (by culture) was 1.34% (vanA prevalence of 1.07% and vanB prevalence of 0.27%). There were 353 specimens positive for the vanA gene by PCR and 330 positive by conventional culture (Tables 1 and 2). The overall prevalence of VRE was 1.34% (vanA prevalence of 1.07% and vanB prevalence of 0.27%). There were 353 specimens positive for the vanA gene by PCR and 330 positive by conventional culture (Tables 1 and 2). Compared to conventional culture, PCR of the vanA gene had a sensitivity of 73.3% and a specificity of 99.6%. The positive predictive value and negative predictive value were calculated as 68.5% and 99.7%, respectively. There were 4,925 specimens positive for vanB by PCR and 82 positive by conventional culture (Tables 1 and 2). Compared to conventional culture, PCR of the vanB gene had
a sensitivity of 85.4% and a specificity of 83.9%. The positive predictive value and negative predictive value were calculated as 1.42% and 99.9%, respectively.

In our study, vanB PCR has a specificity of 83.9% with a positive predictive value of only 1.42%. The poor specificity can be explained by the high prevalence of vanA genes not associated with VRE from human rectal swabs. Graham et al. demonstrated high rates of nonenterococcal vanB carriage in hemodialysis patients (45%), community adults (63%), and children (27%) (6). This is attributed to the presence of gut anaerobes carrying the vanB-containing transposons Tn5382 and Tn1549 (1, 2, 4). Clostridium species, Eggerthella lenta, and Ruminococcus species isolated from rectal specimens were all demonstrated to carry the vanB operon associated with the Tn5382 and Tn1549 element (1, 2, 4). A limitation of this study is that an enrichment broth was not used and may have affected the sensitivity of VRE isolation by culture. While some studies have demonstrated that broth enrichment may increase the VRE detection by 10 to 30% (10, 12), the number of specimens positive for vanB by PCR yet negative by culture far exceeds what one would expect even if an enrichment broth was used.

Relying on a positive vanB PCR result alone would result in the unnecessary utilization of hospital resources and infection control prevention measures for patients who are not harboring VRE. Hence, a positive vanB PCR result is poorly predictive and requires culture to differentiate VRE-positive patients from VRE-negative (i.e., PCR false-positive) patients.

### REFERENCES


