JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2009, p. 4136–4137 Vol. 47, No. 12
0095-1137/09/$12.00 doi:10.1128/JCM.01547-09

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

Anton Mak,[1] Mark A. Miller,[2]* George Chong,[3] and Yury Monczak[3]

Department of Microbiology, McGill University Health Centre, Montreal, Quebec, Canada[1]; Department of Microbiology, Jewish General Hospital, Montreal, Quebec, Canada[2]; and Molecular Pathology, Department of Pathology, Jewish General Hospital, Montreal, Quebec, Canada[3]

Received 11 August 2009/Returned for modification 16 September 2009/Accepted 12 October 2009

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 resistance (Van) gene operons. *vanA* and *vanB* remain 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.

(This paper was presented in part at the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy-Infectious Diseases Society of America 46th Annual Meeting, Washington, DC, 2008.)

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* (*vanB* or *vanB2* or *vanB3*). Concurrently, 100 μl of the saline from the rectal swab was plated onto Enterococcosel 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. Enterococcal isolates were identified with a compatible Gram stain, negative catalase reaction, positive pyrrolidonyl arylamidase test, and growth in 6.5% sodium chloride. Testing of 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, Van 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). 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

* Corresponding author. Mailing address: Division of Clinical Microbiology, Jewish General Hospital, 3755 chemin de la Côte-Ste-Catherine, Montreal, Quebec, Canada H3T 1E2. Phone: (514) 340-8294. Fax: (514) 340-7546. E-mail: mmiller@lab.jgh.mcgill.ca.

▽ Published ahead of print on 21 October 2009.

TABLE 1. Distribution of culture and PCR results of *vanA* and *vanB* testing

VRE test result	No.
Specimens received	30,835
Patients with at least one specimen taken	12,983
Specimens positive for *vanA* by PCR	353
Specimens positive for *vanA* by culture	330
Patients positive by PCR for *vanA*	163
Patients identified with *vanA* by culture	160
Specimens positive for *vanB* by PCR	4,925
Specimens positive for *vanB* by culture	82
Patients positive by PCR for *vanB*	2,897
Patients identified with *vanB* by culture	43
Specimens negative by PCR for VRE (*vanA* and *vanB*)	25,089
Patients negative for VRE by PCR	11,648
Specimens for which PCR was not performed	468

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 *vanB* 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 Tn*5382* and Tn*1549* (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 Tn*5382* and Tn*1549* 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.

TABLE 2. Comparison of selective culture with PCR for *vanA* and *vanB* genes

PCR and result type	No. of results by culture		
	Positive	Negative	Total
PCR *vanA*			
Positive	242	111	353
Negative	88	29,926	30,014
Total	330	30,037	30,367
PCR *vanB*			
Positive	70	4,855	4,925
Negative	12	25,430	25,442
Total	82	30,285	30,367

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

1. **Ballard, S. A., E. A. Grabsch, P. D. R. Johnson, and M. L. Grayson.** 2005. Comparison of three primer sets for identification of VanB gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by *vanB*-containing anaerobic bacilli. Antimicrob. Agents Chemother. **49:**77–81.
2. **Ballard, S. A., K. K. Pertile, M. Lim, P. D. R. Johnson, and M. L. Grayson.** 2005. Molecular characterization of *vanB* elements in naturally occurring gut anaerobes. Antimicrob. Agents Chemother. **49:**1688–1694.
3. **Condon, S., R. Manji, F. Zhang, and C. C. Ginochio.** 2008. Evaluation of a real-time PCR assay for the presumptive identification of vancomycin-resistant enterococci in rectal specimens, abstr. D-321, p. 227. Abstr. 48th Annu. Intersci. Conf. Antimicrob. Agents Chemother. (ICAAC)-Infect. Dis. Soc. Am. (IDSA) 46th Annu. Meet. American Society for Microbiology and Infectious Diseases Society of America, Washington, DC.
4. **Domingo, M. C., A. Huletsky, A. Bernal, R. Giroux, D. K. Boudreau, F. J. Picard, and M. G. Bergeron.** 2005. Characterization of a Tn5382-like transposon containing the vanB2 gene cluster in a *Clostridium* strain isolated from human faeces. J. Antimicrob. Chemother. **55:**466–474.
5. **Gold, H. S.** 2001. Vancomycin resistant enterococci: mechanism and clinical observations. Clin. Infect. Dis. **33:**210–219.
6. **Graham, M., S. A. Ballard, E. A. Grabsch, P. D. Johnson, and M. L. Grayson.** 2008. High rates of fecal carriage of nonenterococcal *vanB* in both children and adults. Antimicrob. Agents Chemother. **52:**1195–1197.
7. **Koh, T. H., R. N. Deepak, S. Y. Se-Thoe, R. V. T. P. Lin, and E. S. C. Koay.** 2007. Experience with the Roche LightCycler VRE detection kit during a large outbreak of vanB2/B3 vancomycin-resistant *Enterococcus faecium*. J. Antimicrob. Chemother. **60:**182–183.
8. **Krcmery, V., Jr., S. Spanik, and J. Trupl.** 1996. First report of vancomycin resistant *Streptococcus mitis* in a leukemic patient after prophylaxis with quinolones and during treatment with vancomycin. J. Chemother. **8:**325–326.
9. **Morgan, M. A., E. Youssef, and G. Maglanoc.** 2008. Evaluation of BD GeneOhm VanR assay for the detection of vancomycin-resistant enterococcus from perianal swabs, abstr. D-322, p. 227. Abstr. 48th Annu. Intersci. Conf. Antimicrob. Agents Chemother. (ICAAC)-Infect. Dis. Soc. Am. (IDSA) 46th Annu. Meet. American Society for Microbiology and Infectious Diseases Society of America, Washington, DC.
10. **Novicki, T. J., J. M. Schapiro, B. K. Ulness, A. Sebeste, L. Busse-Johnston, K. M. Swanson, S. R. Swanzy, W. Leisenring, and A. P. Limaye.** 2004. Convenient selective differential broth for isolation of vancomycin-resistant enterococcus from fecal material. J. Clin. Microbiol. **42:**1637–1640.
11. **Poyart, C., C. Pierre, G. Quesne, B. Pron, P. Berche, and P. Trieu-Cuot.** 1997. Emergence of vancomycin resistance in the genus *Streptococcus*: characterization of a VanB transferable determinant in *Streptococcus bovis*. Antimicrob. Agents Chemother. **41:**24–29.
12. **Roger, M., M. C. Faucher, P. Forest, P. St.-Antoine, and F. Coutlee.** 1999. Evaluation of a *vanA*-specific PCR assay for detection of vancomycin-resistant *Enterococcus faecium* during a hospital outbreak. J. Clin. Microbiol. **37:**3348–3349.
13. **Stamper, P. D., M. Cai, C. Lema, K. Eskey, and K. C. Carroll.** 2007. Comparison of the BD GeneOhm VanR assay to culture for identification of vancomycin-resistant enterococci in rectal and stool specimens. J. Clin. Microbiol. **45:**3360–3365.
14. **Young, H. L., S. A. Ballard, P. Roffrey, and M. L. Grayson.** 2007. Direct detection of VanB2 using the Roche LightCycler vanA/B detection assay to indicate vancomycin resistant enterococcal carriage—sensitive but not specific. J. Antimicrob. Chemother. **59:**809–810.