JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2006, p. 1578–1580 Vol. 44, No. 4
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.4.1578–1580.2006

A 24-Hour Screening Protocol for Identification of Vancomycin-Resistant *Enterococcus faecium*

Steven J. Drews,[1,2] Grant Johnson,[1] Farhad Gharabaghi,[1] Margaret Roscoe,[1] Anne Matlow,[2,3] Raymond Tellier,[1,2] and Susan E. Richardson[1,2]*

Division of Microbiology, Hospital for Sick Children, Toronto, ON, Canada[1]; Department of Pathobiology and Laboratory Medicine, University of Toronto, Toronto, ON, Canada[2]; and Department of Paediatrics, Hospital for Sick Children, Toronto, ON, Canada[3]

Received 12 October 2005/Returned for modification 29 October 2005/Accepted 14 January 2006

We describe a 24-h protocol for the identification of patients who are positive for vancomycin-resistant *Enterococcus faecium* (VRE), using stool and rectal swab samples and VRE screening broth, automated DNA extraction, and real-time PCR for *vanA* and *vanB* genes. Compared to conventional screening methods, this protocol had a high sensitivity and specificity and a negative predictive value.

Vancomycin-resistant enterococci (VRE) are antibiotic-resistant colonizers of the gastrointestinal tract that cause nosocomial outbreaks of both colonization of the gastrointestinal tract and infection at various sites (2, 4, 6, 7). Our pediatric institution has a low prevalence of VRE, and we use conventional VRE screening methods, which require up to 5 days to identify VRE-positive patients (9) and 3 days to identify VRE-negative patients. Other attempts to expedite VRE detection have included the use of 24- to 36-h selective broth cultures (9) and real-time PCR assays, using either swabs or broth for the identification of *vanA* and *vanB* genes encoding vancomycin resistance determinants (9, 11). Preliminary work in our laboratory indicated that direct testing of rectal swabs with the Roche LightCycler VRE detection kit using DNA from our automated extraction protocol was inhibited in 50% of the samples tested. In light of these findings, the purpose of this study was to validate a 24-h protocol for the confirmation of VRE-negative and VRE-positive patients by the use of stool or rectal swab samples screened with a VRE-selective broth for 18 h prior to automated DNA extraction and real-time PCR.

This study was initiated during an outbreak of infection with *vanA*-positive vancomycin-resistant *Enterococcus faecium* at a quaternary-care pediatric institution. A total of 1,863 samples were screened for VRE, and 34 patients were found to be colonized with vancomycin-resistant *E. faecium*. Twenty-six stool and 26 rectal swab samples were collected from 34 VRE culture-positive patients. We randomly chose 55 stool samples and 54 rectal swabs from patients defined as VRE contacts who were admitted to a specific floor at the same time as a VRE-positive person. Rectal swabs were transported in Amies agar gel medium with charcoal (Copan Venturi Transystem swab, Copan, Italy). Amies agar gel medium without charcoal (Copan Venturi Transystem swab, Copan, Italy) was used for VRE-positive mock samples. Stool samples were collected in 100-ml Starplex LeakBuster specimen containers (Starplex Scientific, Inc., Etobicoke, ON, Canada). Direct culture and enrichment broth cultures were carried out using the same swab. Stool samples from patients were frozen at −70°C until inoculated into the VRE-selective broth. Swabs were stored at 4°C until the next available PCR run. The positive and negative control VRE strains were *Enterococcus faecalis* strain ATCC 51299 and *E. faecalis* strain ATCC 29212, respectively.

Rectal swab and stool samples were inoculated onto screening plates consisting of Difco mEnterococcus agar with 6 μg/ml vancomycin (Becton Dickinson and Company, Sparks, MD). Rectal swabs and approximately 5 mg of stool were inoculated into 3 ml of a proprietary VRE-selective broth containing vancomycin and esculin (Oxoid, Nepean, ON, Canada). VRE screening plates were incubated for 72 h at 35°C under aerobic conditions. Stationary VRE-selective broth cultures were incubated for 18 h at 35°C in an aerobic incubator, and all broth samples were scored as clear (possible VRE negative) or black (possible VRE positive) at the end of incubation. To correlate broth culture reactions with PCR results, all positive and negative broth samples were sent for DNA extraction.

To test the hypothesis that broth culture may be more sensitive than conventional solid agar culture or PCR assay, 10 black broths from 10 VRE culture-negative samples were randomly chosen and inoculated onto VRE screening medium. To test the hypothesis that the charcoal within Amies transport buffer may inhibit PCRs, equivalent amounts of VRE from enrichment broth culture were inoculated onto swabs and placed into Amies transport medium with ($n = 20$) or without ($n = 20$) charcoal. Mock samples were stored at 4°C for 24 h and processed according to the protocol described above.

Organism identification and susceptibility testing were carried out as previously described (5, 12) using a BD PHOENIX automated microbiology instrument (BD Canada, Oakville, ON, Canada). DNA was extracted from 200 μl of gravity-settled enrichment broth culture using MagAttract DNA blood M48 kits on a BioRobot M48 workstation (QIAGEN, Mississauga, ON, Canada). 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* (11). PCRs were considered inhibited if internal controls were inhibited in *vanA*- or *vanB*-negative PCR samples or reactions yielded low-amplitude peaks and *vanA*-compatible

* Corresponding author. Mailing address: Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, 555 University Ave., Room 3654, Toronto, Ontario M5G 1X8, Canada. Phone 416-813-5992. Fax: 416-813-6257. E-mail: susan.richardson@sickkids.ca.

TABLE 1. Comparison of conventional culture with a 24-h protocol for identification of VRE in stool samples and rectal swabs

PCR results[a]	Total no. (%) of samples			
	Stool		Swab	
	VRE culture+ (n = 26)	VRE culture− (n = 55)	VRE culture+ (n = 26)	Swab VRE culture− (n = 54)
Positive	26 (100)	0 (0)	25 (96)	0 (0)
Negative	0 (0)	55 (100)	1 (4)	54 (100)

[a] Positive, *vanA* positive; negative, *vanA* negative.

melting curves that could not be analyzed for genotype by the Roche program. DNA was reextracted from swab broth culture samples in which there was PCR inhibition, and the reextracted DNA was diluted 1/10 in distilled water and was rerun using the VRE real-time PCR protocol. Data analysis was done using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Broth screening and culture of stool samples. All VRE culture-positive stool samples (n = 26) produced black (positive) selective broth cultures. Forty-seven of 55 (85%) VRE culture-negative stool samples produced black (positive) selective broth cultures. Compared to standard culture techniques, the VRE-selective broth culture used to predict VRE in stool samples within 18 h had a sensitivity of 100%, a specificity of 15%, and a negative predictive value of 100%.

Broth screening and culture of rectal swab samples. All VRE culture-positive rectal swab samples (n = 26) produced black (positive) selective broth cultures. Fifteen of 54 (28%) VRE culture-negative rectal swab samples produced black (positive) selective broth cultures. Compared to standard culture techniques, the selective broth culture used to predict VRE in rectal swab samples within 18 h had a sensitivity of 100%, a specificity of 72%, and a negative predictive value of 100%. The specificity of the VRE-selective broth using rectal swabs to predict VRE was greater than the specificity using stool samples ($\chi^2 = 36.96$; df = 1; $P < 0.001$).

Broth screening and PCR of stool samples. Compared to conventional culture, the sensitivity, specificity, and negative predictive value of the VRE PCR protocol for selective broth cultures of stool samples were 100% each with no inhibition of PCRs (Table 1).

Broth screening and PCR of rectal swab samples. On initial testing, the sensitivity, specificity, and negative predictive value of the VRE PCR protocol using selective broth cultures of rectal swabs were 84%, 96%, and 93%, respectively. Following reextraction and dilution of inhibited samples, the sensitivity, specificity, and negative predictive value of the VRE PCR protocol using selective broth cultures of rectal swabs were 96%, 100%, and 98%, respectively, compared to conventional culture (Table 1). DNA from one broth culture was *vanA* negative following reextraction and rerunning of the PCR assay. One microliter of broth culture from this PCR-negative sample was found to be positive for *vanA*-positive *E. faecium* by both culture and confirmatory PCR. None of the mock samples on swabs with or without charcoal were inhibited in the PCR.

A comparison of PCR test characteristics for stool samples and rectal swabs showed that on initial testing, VRE-selective broth cultures of rectal swab samples had significantly greater PCR inhibition, requiring repeat DNA extractions and PCRs, than selective broths of stool samples did ($\chi^2 = 6.31$; df = 1; $P = 0.025$) (Table 1). Following reextraction and dilution of inhibited samples, there was no significant difference in PCR inhibition between VRE-selective broth cultures of rectal swab and VRE-selective broth cultures of stool samples ($\chi^2 = 1.02$; df = 1; $P > 0.05$).

We were interested in whether selective broth culture might enrich for VRE in samples identified as VRE negative by conventional culture. However, subculture of 10 black broth cultures from VRE culture-negative stool samples and swabs from patients who previously had cultures positive for VRE failed to identify viable VRE. Of the 10 cultures, nine grew nothing on the screen plates and one grew a *Pediococcus* strain. These broths also tested negative by PCR.

One limitation to this study is that it did not analyze clinical samples containing either vancomycin-resistant *E. faecalis* or VRE with the *vanB* determinant. Further work should be done to validate this protocol for clinical isolates containing these organisms.

Stool and rectal swab samples each have relative advantages and disadvantages for the identification of VRE-positive and -negative patients. Rectal swabs are useful when large numbers of samples are collected because the expense of PCR testing can be avoided in 72% of VRE-negative patients. PCR inhibition with rectal swab testing may be due to patient-dependent factors in the stool, including excess human DNA, chemicals, or heme (8), and may be overcome by diluting extracted DNA prior to PCR testing. In contrast, the poor specificity of stool samples in VRE-selective broth may be due to a larger inoculum of esculin-hydrolyzing organisms than is present in rectal swabs (3). Although it may take more time for a patient to produce a stool sample, the advantage is that stool samples can be collected from patients undergoing chemotherapy, radiation therapy, or stem cell transplantation, conditions under which rectal swabs may be contraindicated (1, 10). In this outbreak, a large percentage of the cases occurred on the oncology and bone marrow transplantation wards, which made the sampling method an important issue. Collecting and freezing a fresh stool sample from such patients until the next PCR testing run still provides a quicker turnaround time than do traditional culture techniques for VRE screening.

This assay identifies VRE-positive and -negative patients within 24 h of selective broth inoculation. During the preparation of this report, an oncology ward in our institution had a small culture-verified VRE outbreak due to *E. faecium* carrying *vanA*. We found that (i) stool samples were easily collected; (ii) PCR inhibition was not seen in broth enrichment samples of stool samples; and (iii) there was 100% agreement between our traditional culture protocol and this newly implemented VRE enrichment culture coupled to a PCR assay, when DNA was extracted from broth cultures of stool samples. We also found that (i) rectal swabs are useful specimens for testing with this protocol and (ii) inhibition can be largely overcome with reextraction and dilution of PCR-inhibited samples.

REFERENCES

1. **Blijlevens, N. M.** 2005. Implications of treatment-induced mucosal barrier injury. Curr. Opin. Oncol. **17:**605–610.

2. **Boyle, J. F., S. A. Soumakis, A. Rendo, J. A. Herrington, D. G. Gianarkis, B. E. Thurberg, and B. G. Painter.** 1993. Epidemiologic analysis and genotypic characterization of a nosocomial outbreak of vancomycin-resistant enterococci. J. Clin. Microbiol. **31:**1280–1285.
3. **Facklam, R. R., and M. D. Collins.** 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27:**731–734.
4. **Frieden, T. R., S. S. Munsiff, D. E. Low, B. M. Willey, G. Williams, Y. Faur, W. Eisner, S. Warren, and B. Kreiswirth.** 1993. Emergence of vancomycin-resistant enterococci in New York City. Lancet **342:**76–79.
5. **Hospital Infection Control Practices Advisory Committee, CDC.** 1995. Recommendations for preventing the spread of vancomycin resistance. Infect. Control Hosp. Epidemiol. **16:**105–113.
6. **Low, D. E., N. Keller, A. Barth, and R. N. Jones.** 2001. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin. Infect. Dis. **32**(Suppl. 2)**:**S133–S145.
7. **McGeer, A. J., and D. E. Low.** 2000. Vancomycin-resistant enterococci. Semin. Respir. Infect. **15:**314–326.
8. **Morata, P., M. I. Queipo-Ortuno, and J. de Dios Colmenero.** 1998. Strategy for optimizing DNA amplification in a peripheral blood PCR assay used for diagnosis of human brucellosis. J. Clin. Microbiol. **36:**2443–2446.
9. **Palladino, S., I. D. Kay, J. P. Flexman, I. Boehm, A. M. G. Costa, E. J. Lambert, and K. J. Christiansen.** 2003. Rapid detection of *vanA* and *vanB* genes directly from clinical specimens and enrichment broths by real-time multiplex PCR assay. J. Clin. Microbiol. **41:**2483–2486.
10. **Prommer, E.** 2005. Management of bleeding in the terminally ill patient. Hematology **10:**167–175.
11. **Sloan, L. M., J. R. Uhl, E. A. Vetter, C. D. Schleck, W. S. Harmsen, J. Manahan, R. L. Thompson, J. E. Rosenblatt, and F. R. Cockerill III.** 2004. Comparison of the Roche LightCycler *vanA*/*vanB* detection assay and culture for detection of vancomycin-resistant enterococci from perianal swabs. J. Clin. Microbiol. **42:**2636–2643.
12. **VRE Working Group Canadian Hospital Epidemiology Committee and Health Canada Division of Nosocomial and Occupational Infections.** 1997. Preventing the spread of vancomycin-resistant enterococci (VRE) in Canada. Can. Commun. Dis. Rep. **23**(Suppl. 8)**:**i–16.