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Comparison of PCR Assay to Culture for Surveillance Detection of Vancomycin-Resistant Enterococci

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Direct multiplex PCR assay using *vanA* and *vanB* primers, which provides rapid results, was more sensitive than culture on selective media for samples collected by rectal swab (20 of 46 versus 8 of 46; $P < 0.001$) or perianal swab (17 of 58 versus 12 of 58; $P = 0.059$) for the detection of gastrointestinal colonization by vancomycin-resistant enterococci.

Since the first report of human infection with vancomycin-resistant enterococci (VRE) in the late 1980s (A. H. Uttley, C. H. Collins, J. Naidoo, and R. C. George, Letter, Lancet **1:**57–58, 1988), VRE have become common in many hospitals in the United States (6). One likely reason why infection control measures in hospitals have not effectively controlled VRE transmission is the unrecognized reservoir of VRE-colonized patients that contributes to transmission (1, 15). Identification and isolation of VRE-colonized patients have reduced the prevalence of VRE colonization both within a facility (7) and for an entire geographic region (10).

Screening patients for gastrointestinal VRE colonization using culture requires 48 to 72 h of incubation and testing (12). Since the median length of stay for hospitalized patients in the United States in 1999 was 5 days (11), culture may not yield results during a patient's hospital stay. However, the results of PCR assays to detect vancomycin resistance genes *vanA* and *vanB* can be obtained within 8 h (12). Thus, the use of PCR to screen patients should enable early implementation of contact isolation precautions, which can improve VRE infection control efforts.

(This work was presented in part at the 11th Annual Scientific Meeting of the Society for Healthcare Epidemiology of America, Toronto, Ontario, Canada, 2001 [S. M. Paule, W. E. Trick, M. Lankford, V. Stosor, R. Cordell, and L. R. Peterson, abstr. 125].)

From June to November 2000 and July to October 2001, patients admitted to Northwestern Memorial Hospital (NMH) who had VRE detected during a previous NMH hospitalization and who had received less than 24 h of antimicrobial therapy were eligible for enrollment. Unless contraindicated or refused, simultaneous rectal and perianal swabs were collected. Samples were obtained using premoistened rayon-tipped swabs (CultureSwab; Becton Dickinson, Sparks, Md.). Institutional Review Board approval was obtained from Northwestern University and the Centers for Disease Control and Prevention. Clinical information was obtained by medical record review.

Swab specimens were suspended in 350 μl of sterile water, and 50-μl aliquots were used for DNA extraction or plated onto Enterococcosel (BBL) medium containing 6 μg of vancomycin (Sigma, St. Louis, Mo.) per ml. Isolates were identified as *Enterococcus faecium* or *Enterococcus faecalis* by standard biochemical methods (5), and their agar dilution susceptibility was tested following National Committee for Clinical Laboratory Standards guidelines (9). DNA was extracted using the MasterPure DNA purification kit (Epicentre Technologies, Madison, Wis.) with the following modifications: 50 μl of lysozyme (100 mg/ml; Sigma) was added to the 50-μl aliquot and incubated at 37°C for 30 min, and 100 μg of proteinase K was used. The final DNA pellet was suspended in 50 μl of sterile water, and 5 μl was used in a 50-μl hot-start PCR mixture containing 0.4 μM (each) primer for the *vanA* and *vanB* genes (4) and 1× Platinum PCR Supermix (Invitrogen, Carlsbad, Calif.) for the samples collected in 2000 or 1× AmpliTaq Gold PCR Mastermix (Applied Biosystems, Foster City, Calif.) for the samples collected in 2001. DNA extraction controls, i.e., water for the negative control and a *vanB*-containing *E. faecium* for the positive control, and PCR controls, i.e., vancomycin-susceptible *E. faecium* for the negative control and *vanA*-containing *E. faecium* for the positive control, were processed during each run. The amplification program consisted of the following: (i) an initial step of 5 min at 95°C; (ii) 30 cycles consisting of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C; and (iii) a final extension step of 5 min at 72°C. The PCR products, 732 bp for *vanA* and 635 bp for *vanB*, were resolved on a 1.5% agarose gel, stained with SYBR green I (Molecular Probes, Eugene, Oreg.), and imaged under UV light. In serial dilution sensitivity analyses using the *vanA*- or *vanB*-containing *E. faecium* control strains, both the Platinum *Taq* PCR and the AmpliTaq Gold PCR were able to detect 20 CFU.

We compared results from PCR to those of culture, segregated by specimen source (e.g., rectal versus perianal) using

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TABLE 1. Comparison of PCR assay to culture on selective media for detecting gastrointestinal VRE colonization by perianal and rectal swab[a]

Swab	Study swab			Previous phenotype[c]	Concordance (%) between the study genotype and previous phenotype
	PCR genotype[b]	Culture phenotype	Concordance (%) between the genotype and phenotype		
Perianal swabs ($n = 58$)[d]					
PCR and culture positive ($n = 11$)	*vanA* ($n = 9$)	VanA ($n = 9$)	100	VanA ($n = 10$)	100
	vanB ($n = 3$)	VanB ($n = 2$)	100	VanB ($n = 2$)	
PCR positive and culture negative ($n = 6$)	*vanA* ($n = 6$)			VanA ($n = 6$)	100
				VanB ($n = 1$)	
PCR negative and culture positive ($n = 1$)		VanA ($n = 1$)		VanA ($n = 1$)	
PCR and culture negative ($n = 40$)				VanA ($n = 30$)	
				VanB ($n = 16$)	
Rectal swabs ($n = 46$)[e]					
PCR and culture positive ($n = 8$)	*vanA* ($n = 6$)	VanA ($n = 6$)	100	VanA ($n = 7$)	100
	vanB ($n = 3$)	VanB ($n = 2$)	100	VanB ($n = 2$)	
PCR positive and culture negative ($n = 12$)	*vanA* ($n = 5$)			VanA ($n = 11$)	50
	vanB ($n = 8$)			VanB ($n = 4$)	
PCR and culture negative ($n = 26$)				VanA ($n = 19$)	
				VanB ($n = 12$)	

[a] Study genotypes and phenotypes and the previous culture phenotype are included.
[b] Both *vanA* and *vanB* genetic elements detected from the same patient were counted for each category.
[c] Both VanA and VanB phenotypes recovered from the same patient were counted for each category. The phenotype from the previous isolate was not available for one participant.
[d] $P = 0.059$ by the McNemar test for paired samples.
[e] $P < 0.001$ by the McNemar test for paired samples.

the McNemar test for paired samples. All analyses were performed using SAS version 8.0 software (SAS Institute, Cary, N.C.).

At least one rectal or perianal swab was evaluated by both PCR and culture for each participant. Of the 59 participants, 63% were white, 53% were male, and the median age was 54 years. The median length of time since the last VRE-positive culture was 258 days (range, 17 to 2,482 days). Among the 58 perianal swabs evaluated by PCR and culture, 11 were positive by both methods, 40 were negative by both methods, 1 was positive by culture only, and 6 were positive by PCR only ($P = 0.059$). Among the 46 rectal swabs, 8 were positive by both methods, 26 were negative by both methods, none were positive by culture only, and 12 were positive by PCR only ($P < 0.001$) (Table 1). For the 11 perianal specimens and the 8 rectal specimens that were positive by both PCR and culture, there was 100% concordance between the PCR genotype, the study culture phenotype, and the previous VRE phenotype. The six PCR-positive, culture-negative perianal specimens all had the *vanA* genotype, which was concordant with their previous VRE phenotype. However, for 6 of the 12 PCR-positive, culture-negative rectal specimens, the PCR genotype was discordant with the patients' previous VRE phenotype and 5 of the 6 specimens were detected to have the *vanB* gene. Possible explanations for *vanB* detection by PCR from culture-negative rectal swabs include the following: PCR may detect *vanB* from nonviable VRE isolates, or PCR may be detecting *vanB* genetic elements from nonenterococcal organisms (13).

Other investigators have reported poor sensitivity of rectal swab cultures at low fecal densities of VRE (2, 8) and that PCR is a sensitive method for VRE detection by rectal swab (12). In our study, stool cultures were not evaluated; it is possible that PCR-positive, culture-negative patients have low fecal densities of VRE. Since low fecal densities of VRE decrease the efficiency of environmental contamination (2), the importance of PCR detection of VRE from culture-negative patients is unknown. However, it is known that the density of VRE in an individual is variable and dependent on recent antimicrobial exposure (2, 3).

A proven effective strategy to minimize transmission of VRE in healthcare facilities is detection of colonized patients and initiation of contact isolation precautions (7, 10). We found that among patients previously colonized by VRE, PCR yielded positive results more often than did culture for both rectal and perianal swabs. In addition to enhanced sensitivity, another potential benefit of VRE detection using PCR is the difference in time necessary to obtain results. Potential disadvantages of using only PCR to detect VRE colonization include the unavailability of isolates for molecular epidemiologic investigation (14), the requirements to perform PCR, and the potential to unnecessarily place patients on contact isolation precautions because of a false-positive result (e.g., the genetic element was detected from a nonenterococcal organism or a nonviable enterococcus). Culturing PCR-positive swabs can solve many of these issues.

In conclusion, we found that among previously colonized patients, use of PCR on perianal swabs is a sensitive and accurate method to detect gastrointestinal VRE colonization.

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REFERENCES

1. **Byers, K. E., A. M. Anglim, C. J. Anneski, T. P. Germanson, H. S. Gold, L. J. Durbin, B. M. Simonton, and B. M. Farr.** 2001. A hospital epidemic of vancomycin-resistant enterococcus: risk factors and control. Infect. Control Hosp. Epidemiol. **22:**140–147.

2. **D'Agata, E. M., S. Gautam, W. K. Green, and Y. W. Tang.** 2002. High rate of false-negative results of the rectal swab culture method in detection of gastrointestinal colonization with vancomycin-resistant enterococci. Clin. Infect. Dis. **34:**167–172.
3. **Donskey, C. J., T. K. Chowdhry, M. T. Hecker, C. K. Hoyen, J. A. Hanrahan, A. M. Hujer, R. A. Hutton-Thomas, C. C. Whalen, R. A. Bonomo, and L. B. Rice.** 2000. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. N. Engl. J. Med. **343:**1925–1932.
4. **Dutka-Malen, S., S. Evers, and P. Courvalin.** 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. **33:**24–27.
5. **Facklam, R. R., D. F. Sham, and L. M. Teixeira.** 1999. Enterococcus, p. 297–305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
6. **Hayden, M. K.** 2000. Insights into the epidemiology and control of infection with vancomycin-resistant enterococci. Clin. Infect. Dis. **31:**1058–1065.
7. **Jochimsen, E. M., L. Fish, K. Manning, S. Young, D. Singer, R. Baker, and W. Jarvis.** 1999. Control of vancomycin-resistant enterococci at a community hospital: efficacy of patient and staff cohorting. Infect. Control Hosp. Epidemiol. **20:**106–109.
8. **Landman, D., J. M. Quale, E. Oydna, B. Willey, V. DiTore, M. Zaman, K. Patel, G. Saurina, and W. Huang.** 1996. Comparison of five selective media for identifying fecal carriage of vancomycin-resistant enterococci. J. Clin. Microbiol. **34:**751–752.
9. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. **Ostrowsky, B. E., W. E. Trick, A. H. Sohn, S. B. Quirk, S. Holt, L. A. Carson, B. C. Hill, M. J. Arduino, M. J. Kuehnert, and W. R. Jarvis.** 2001. Control of vancomycin-resistant enterococcus in health care facilities in a region. N. Engl. J. Med. **344:**1427–1433.
11. **Popovic, J. R.** 2001. 1999 National hospital discharge survey: annual summary with detailed diagnosis and procedure data. Vital and Health Statistics, series 13, no. 151. Department of Health and Human Services publication no. (PHS) 2001–1722. National Center for Health Statistics, Centers for Disease Control and Prevention, Department of Health and Human Services, Hyattsville, Md.
12. **Satake, S., N. Clark, D. Rimland, F. S. Nolte, and F. C. Tenover.** 1997. Detection of vancomycin-resistant enterococci in fecal samples by PCR. J. Clin. Microbiol. **35:**2325–2330.
13. **Stinear, T. P., D. C. Olden, P. D. Johnson, J. K. Davies, and M. L. Grayson.** 2001. Enterococcal *van*B resistance locus in anaerobic bacteria in human faeces. Lancet **357:**855–856.
14. **Tenover, F. C., R. D. Arbeit, R. V. Goering, et al.** 1997. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Infect. Control Hosp. Epidemiol. **18:**426–439.
15. **Trick, W. E., M. J. Kuehnert, S. B. Quirk, M. J. Arduino, S. M. Aguero, L. A. Carson, B. C. Hill, S. N. Banerjee, and W. R. Jarvis.** 1999. Regional dissemination of vancomycin-resistant enterococci resulting from interfacility transfer of colonized patients. J. Infect. Dis. **180:**391–396.