ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2005, p. 77–81 Vol. 49, No. 1
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.1.77–81.2005

Comparison of Three PCR Primer Sets for Identification of *vanB* Gene Carriage in Feces and Correlation with Carriage of Vancomycin-Resistant Enterococci: Interference by *vanB*-Containing Anaerobic Bacilli

S. A. Ballard,[1] E. A. Grabsch,[2] P. D. R. Johnson,[1,3] and M. L. Grayson[1,3,4]*

Infectious Diseases[1] and Microbiology[2] Departments, Austin Health, Department of Medicine, University of Melbourne,[3] and Department of Epidemiology and Preventive Medicine, Monash University,[4] Melbourne, Australia

Received 24 June 2004/Returned for modification 26 August 2004/Accepted 18 September 2004

We assessed the sensitivities and specificities of three previously described PCR primers on enrichment broth cultures of feces for the accurate detection of fecal carriage of vancomycin-resistant enterococci (VRE). In addition, we investigated specimens that were *vanB* PCR positive but VRE culture negative for the presence of other *vanB*-containing pathogens. Feces from 59 patients (12 patients carrying *vanB Enterococcus faecium* strains and 47 patients negative for VRE carriage) were cultured for 36 h in aerobic brain heart infusion (BHI) broth, anaerobic BHI (AnO_2BHI) broth, or aerobic Enterococcosel (EC) broth. DNA was extracted from the cultures and tested for the presence of *vanB* by using the PCR primers of Dutka-Malen et al. (S. Dutka-Malen, S. Evers, and P. Courvalin, J. Clin. Microbiol. 33:24–27, 1995), Bell et al. (J. M. Bell, J. C. Paton, and J. Turnidge, J. Clin. Microbiol. 36:2187–2190, 1998), and Stinear et al. (T. P. Stinear, D. C. Olden, P. D. R. Johnson, J. K. Davies, and M. L. Grayson, Lancet 357:855–856, 2001). The sensitivity (specificity) of PCR compared with the results of culture on BHI, AnO_2BHI, and EC broths were 67% (96%), 50% (94%), and 17% (100%), respectively, with the primers of Dutka-Malen et al.; 92% (60%), 92% (45%), and 92% (83%), respectively, with the primers of Bell et al.; and 92% (49%), 92% (43%), and 100% (51%) respectively, with the primers of Stinear et al. The primers of both Bell et al. and Stinear et al. were significantly more sensitive than those of Dutka-Malen et al. in EC broth ($P = 0.001$ and $P < 0.001$, respectively). The poor specificities for all primer pairs were due in part to the isolation and identification of six anaerobic gram-positive bacilli, *Clostridium hathewayi* ($n = 3$), a *Clostridium innocuum*-like organism ($n = 1$), *Clostridium bolteae* ($n = 1$), and *Ruminococcus lactaris*-like ($n = 1$), from five fecal specimens that were *vanB* positive but VRE culture negative. All six organisms were demonstrated to contain a *vanB* gene identical to that of VRE. *VanB*-containing bowel anaerobes may result in false-positive interpretation of PCR-positive fecal enrichment cultures as VRE, regardless of the primers and protocols used.

Minimization of the risk of colonization and infection by vancomycin-resistant enterococci (VRE) is a major infection control goal in Australia, as elsewhere. However, the prolonged time that it takes to obtain results by routine microbiological screening techniques often results in delays in implementation of appropriate infection control measures, such as separating patients into cohorts (12, 21). Six different vancomycin resistance gene (*van*) operons (*vanA*, *vanB*, *vanC*, *vanD*, *vanE*, and *vanG*) are known to mediate glycopeptide resistance, with three alleles described for the *vanB* ligase gene (*vanB1*, *vanB2*, and *vanB3*) (6, 16, 23). The most commonly recognized pathogenic genotype in Australia is *vanB*, the *vanB2* allele of which predominates (A. Berry, H. Sundqvist, J. Bell, and J. Turnidge, Abstr. 3rd Annu. Sci. Meet. Aust. Soc. Antimicrob., abstr. 22, Feb. 2002).

Present PCR protocols for the direct detection of VRE in clinical specimens or enrichment broth cultures of these specimens rely on the detection of *van* genes, specifically, *vanA* or *vanB* (18, 22, 25, 28, 29). These methods are not dependent on the identity of the host organism, and as such, the identification of VRE can only be inferred, as the specimen contains a mixed bacterial population. Since the *vanB* gene has been described in *Streptococcus* species and more recently in *Eggerthella lenta* and three *Clostridium* species isolated from human feces, the presence of *vanB* in feces does not necessarily imply the presence of VRE (19, 27, 31). For this reason, we assessed the sensitivities and specificities of three frequently used primers under different growth and PCR conditions for the accurate identification of VRE in fecal specimens from patients for whom knowledge about their VRE carriage status was known.

(This work was presented in part at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., September 2003 [S. A. Ballard, E. A. Grabsch, S. Xie, P. D. R. Johnson, and M. L. Grayson, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-1890, 2003].)

MATERIALS AND METHODS

Clinical specimens and bacterial strains. Fecal samples were collected from 59 patients who were previously well characterized with regard to the presence or absence of fecal VRE carriage. Fecal specimens were collected from 53 hemodialysis patients; of these, 6 patients were known to be fecally colonized

* Corresponding author. Mailing address: Infectious Diseases Department, Austin Hospital, Austin Health, Studley Rd., Heidelberg, VIC 3084, Australia. Phone: (613) 9496-6676. Fax: (613) 9496-6677. E-mail: Lindsay.Grayson@austin.org.au.

with *vanB E. faecium*, while 47 patients had repeatedly been shown to be VRE negative. Feces were also collected from six nonhemodialysis patients at our hospital who were known to be fecally colonized with VRE (all with *vanB E. faecium*) as a result of tests with at least two recent consecutive fecal specimens. Thus, all 12 VRE-colonized patients carried *vanB E. faecium*. All fecal specimens were collected and then stored at −80°C. Control enterococcal strains used for PCR were an *Enterococcus faecalis* strain containing *vanB* (ATCC 51299), a vancomycin-sensitive *E. faecalis* strain (ATCC 29212), and clinical isolates of *vanA E. faecium* and *vanB E. faecium*, each of which was obtained at our institution.

Enrichment broth cultures of feces and isolation of VRE. All fecal specimens were assessed for the presence of VRE both by direct culture on agar and by culture in enrichment broth. Enrichment broth cultures of thawed fecal specimens were obtained by the following protocol. A sterile 50-μl plastic loopful of fecal material was used to inoculate each of the following broths (6 ml each): brain heart infusion (BHI) broth and Enterococcosel (EC) broth (both of which were incubated aerobically) and BHI broth with 0.5% yeast extract, 0.1% sodium thioglycolate, 0.25% glucose, and 0.5 μg of hemin ml^{-1} (AnO_2BHI broth; which was incubated anaerobically). All broth cultures were incubated at 35°C for 36 h. Subsequently, all VRE culture-positive specimens were recultured by the same method, but with assessment at both 17 and 36 h.

Isolation of VRE was performed as follows. All fecal specimens and enrichment broth cultures were plated on EC agar containing vancomycin at 6 μg/ml and were incubated at 35°C for 48 to 72 h. Esculin-positive colonies were investigated as described previously (21). The identities of isolates provisionally identified as *Enterococcus faecium* or *E. faecalis* were confirmed by multiplex PCR for the D-Ala–D-Ala ligase gene (7). The glycopeptide resistance genotype was determined by multiplex PCR for *vanA*, *vanB*, and *vanC* (2).

Detection of *van* genes in enrichment broth cultures of feces. DNA was extracted from a 500-μl volume of fecal enrichment broth as described by Stinear et al. (30). DNA samples were frozen at −20°C until they were required. A 5-μl volume of DNA was tested by PCR for *vanA* and *vanB* in a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 2 mM $MgCl_2$; 200 μM each dATP, dTTP, dGTP, and dCTP; 0.25 μM each primer; and 0.25 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, by Roche Molecular Systems, Inc., Branchburg, N.J.). PCR cycling was performed on a GeneAmp2700 thermal cycler (Applied Biosystems). Known positive and negative controls (including multiple controls consisting of sterile water [i.e., no DNA]) were included in each PCR run. The PCR products were resolved by electrophoresis on agarose-Tris-acetate-EDTA gels containing 0.5 μg of ethidium bromide per ml. The gels were visualized and photographed with a Fluor-S MultiImager and Quantity One quantitation software (version 4; Bio-Rad Laboratories, Hercules, Calif.).

The following three different primer sets were assessed. The PCR protocol of Stinear et al. (31) was performed with primers VanB-P1 and VanB-P2, designed for the detection of *vanB*; the primers and PCR protocol are referred to as "Stinear." The cycling conditions for the Stinear protocol were 95°C for 10 min, followed by 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min for 5 cycles; 94°C for 20 s, 58°C for 30 s, and 72°C for 40 s for 35 cycles; and 72°C for 5 min (T. Stinear, personal communication). The PCR protocol of Bell et al. (2) was performed with primers VanABF, VanAR, and VanBR, designed for the detection of *vanA* and *vanB* by multiplex PCR; the primers and PCR protocol are referred to as "Bell." The cycling conditions were 95°C for 10 min, followed by 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min for 40 cycles and 72°C for 5 min (J. Bell, personal communication). The PCR protocol of Dutka-Malen et al. (7) was performed with primers, A_1, A_2, B_1, and B_2, designed for the detection of *vanA* and *vanB*; the primers and PCR protocol are referred to as "Dutka-Malen." The exception to the Dutka-Malen protocol was that 0.5 μM each forward and reverse primers was used. The cycling conditions were as follows: 95°C for 10 min, followed by 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min for 40 cycles and 72°C for 5 min (7). This protocol was repeated by using an annealing temperature of 50°C because of the poor sensitivity obtained by the original method.

Isolation of anaerobes. To optimize the isolation of vancomycin-resistant anaerobes from *vanB*-positive, VRE culture-negative feces, a swab of fecal material was subcultured in 6-ml volumes of AnO_2BHI broth containing vancomycin at 10 μg/ml and incubated anaerobically at 35°C for 48 h, as described above. These cultures were then serially diluted in AnO_2BHI broth, and 100-μl aliquots were plated onto horse blood agar containing vancomycin at 6 μg/ml (HBA-V) and colistin-nalidixic acid blood agar to obtain single colonies. The plates were incubated anaerobically at 35°C for 48 h. Single colonies from both media were assessed for purity on HBA-V. Isolates were investigated by Gram staining, and their identities were determined by sequencing of their 16S rRNA genes. The presence of *vanB* was tested by PCR with the Bell primers, and the gene was sequenced for confirmation (see below).

DNA sequencing and analysis. DNA for sequencing was prepared from pure isolates of anaerobes by using the DNeasy Tissue kit (Qiagen Pty. Ltd., Clifton Hill, Victoria, Australia) and the modified protocol for isolation of genomic DNA from gram-positive bacteria, as described in the instructions of the manufacturer. A 1,376-bp region of the 16S rRNA gene was generated with the primers described by Gurtler et al. (13), and a 2,917-bp region of the *vanB* operon encompassing the *vanB* ligase gene was generated with primers 5′-TGC TTCCAATGAGACGGGCG-3′ and 5′-CTTTGTGCCGATGATGCGAT-3′ by PCR with the Expand Long Template PCR system (Roche Applied Science, Mannheim, Germany). PCR elongation times and temperatures were adjusted according to the expected size of the product and the nucleotide sequences of the primers, respectively, as recommended by the manufacturer. The PCR products to be sequenced were purified with a QIAquick PCR purification kit (Qiagen Pty. Ltd.). The sequences of the PCR products were determined by cycle sequencing with the same primers used for the PCR and the ABI Prism BigDye Terminator (version 3.0) Ready Reaction Cycle Sequencing kit (Applied Biosystems) with an ABI Prism 3100 genetic analyzer. The chromatograms were read and the contiguous sequences were constructed with Vector NTI Advance software (version 8.0; Informax Inc., Bethesda, Md.). The sequences were compared for homology with sequences in the GenBank, EMBL, DDBJ, and PDB databases by using the BLASTN local alignment search tool (1) and the National Center for Biotechnology Information website (www. ncbi.nlm.nih.gov). The sequences of the *vanB* ligase gene were aligned by using the AlignX module of Vector NTI Advance software (version 8.0) and the CLUSTAL W algorithm (33).

Statistical analysis. Statistical comparisons of the sensitivity and the specificity of each primer for the identification of the presence of VRE were undertaken by chi-square analysis.

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S rRNA genes from isolates MLG856-1, MLG856-2, MLG480, MLG080-1, MLG080-3, MLG392, and MLG661 have been submitted to GenBank and given consecutive accession numbers AY653230 to AY653236, respectively. The nucleotide sequences of the *vanB* ligase genes from these isolates have also been submitted to GenBank and given the consecutive accession numbers AY655710 to AY655716, respectively.

RESULTS

A total of 59 fecal specimens, 12 of which were VRE culture positive and 47 of which were VRE culture negative, were examined for the presence of *vanB* by PCR following enrichment broth culture. The sensitivity and specificity of each of the three PCR primer sets for the accurate detection of the presence of fecal VRE carriage with each of the three enrichment broth cultures are shown in Table 1. The Stinear and Bell primers were more sensitive than the Dutka-Malen primers for the detection of VRE under all growth conditions assessed, although this difference was statistically significant only in EC broth ($P < 0.001$ and $P = 0.001$, respectively; Table 1). The optimal balance between sensitivity and specificity appeared to be best achieved with the Bell primers for the fecal cultures in EC broth. Notably, the sensitivity of the Bell primer and PCR protocol did not change when EC broth cultures incubated for only 17 h were used (data not shown). In contrast, the Dutka-Malen primer set appeared to be less sensitive, even when the annealing temperature used in the PCR was decreased from 54 to 50°C.

Both the Bell and Stinear primer sets detected *vanB* genes in a large number of fecal specimens that were culture negative for VRE. This was especially notable when AnO_2BHI enrichment broth was used, with which 26 and 27 of the 47 VRE culture-negative specimens had detectable *vanB* genes when these two primer sets were used, respectively. Attempts were made to isolate the source of the *vanB* signal in these 27 specimens. For 18 specimens the *vanB* gene signal was lost

TABLE 1. Sensitivity and specificity of *vanB* PCR protocols for detection of VRE in feces cultured in enrichment broth for 36 h

Primer and PCR protocol	BHI broth cultures				AnO_2BHI broth cultures				EC broth cultures			
	No. of PCR-positive specimens[a]		Sensitivity (%)	Specificity (%)	No. of PCR-positive specimens		Sensitivity (%)	Specificity (%)	No. of PCR-positive specimens		Sensitivity (%)	Specificity (%)
	VRE + (n = 12)	VRE − (n = 47)			VRE + (n = 12)	VRE − (n = 47)			VRE + (n = 12)	VRE − (n = 47)		
Stinear	11	24	92[b,c]	49	11	27	92[b]	43	12	23	100[d,e]	51[f]
Bell	11	19	92	60	11	26	92[b]	45	11	8	92[g,h]	83[i,j]
Dutka-Malen	8	2	67[c]	96[k]	6	3	50[b]	94[k]	2	0	17[d,g]	100[f,i]
Dutka-Malen[l]	6	0	50[b]	100[k]	6	2	50[b]	96[k]	4	1	33[e,h]	98[f,j]

[a] Number of specimens giving the expected PCR product. VRE +, VRE positive; VRE −, VRE negative.
[b] Difference not statistically significant ($P = 0.07$).
[c] Difference not statistically significant ($P = 0.32$).
[d] Stinear primers significantly more sensitive than Dutka-Malen primers ($P < 0.001$).
[e] Stinear primers significantly more sensitive than Dutka-Malen primers ($P = 0.002$).
[f] Dutka-Malen primers significantly more specific than Stinear primers ($P < 0.001$).
[g] Bell primers significantly more sensitive than Dutka-Malen primers ($P = 0.001$).
[h] Bell primers significantly more sensitive than Dutka-Malen primers ($P = 0.01$).
[i] Dutka-Malen primers significantly more specific than Bell primers ($P = 0.01$).
[j] Dutka-Malen primers significantly more specific than Bell primers ($P = 0.035$).
[k] Dutka-Malen primers significantly more specific than either Stinear or Bell primers ($P < 0.001$).
[l] Dutka-Malen primer and PCR protocol repeated with an annealing temperature of 50°C.

when the positive broth was further subcultured in AnO_2BHI broth under anaerobic conditions. For four specimens, the *vanB* signal persisted after this subculture, but no organisms containing *vanB* could be isolated. A total of seven different *vanB*-containing organisms were isolated from the remaining five *vanB* PCR-positive, VRE culture-negative specimens (Table 2). For one specimen, an *E. faecium* isolate not previously isolated by routine culture methods and an isolate closely related to *Clostridium hathewayi* (98% 16S rRNA gene sequence identity) were identified. Of the remaining five organisms isolated, two were related to *C. hathewayi* (98% 16S rRNA gene identity), one was related to *Clostridium bolteae* (98% 16S rRNA gene identity), and one was most likely a new species with only 91% 16S rRNA gene identity to *Clostridium innocuum*. The remaining isolate was a gram-variable coccobacillus with 91% identity by 16S rRNA gene analysis to the *Ruminococcus lactaris* 16S rRNA gene sequence (Table 2). The entire *vanB* ligase genes from all seven isolates were sequenced. For six of these isolates the *vanB* ligase gene sequence was 99% identical to the *vanB2* sequences of *E. faecium* C68 (3) and *E. faecalis* 268-10 (11), while for MLG480 the *vanB* ligase gene sequence was 99% identical to the *vanB1* sequence of *E. faecalis* V583 (9) and only 97% identical to the *vanB2* sequence.

DISCUSSION

The rapid detection of VRE colonization may have important infection control implications in centers where VRE-colonized patients are separated into cohorts to minimize nosocomial transmission (12, 21). Although conventional culture methods for the detection of VRE may be sensitive, they frequently take 3 to 5 days to confirm the presence or absence of VRE (5, 8, 14, 18, 25, 28). A number of investigators have attempted the direct detection of *van* genes in either feces or enrichment broth cultures of feces as a surrogate means of identifying VRE carriage, with variable results (18, 22, 24, 25, 28). The sensitivities and specificities of such methods depend on the type of *van* genotype, the PCR primers used, and the nature of the enrichment broth.

In our study we found that for the detection of *vanB* VRE, PCR with the Bell primers with DNA extracted from 36-h EC broth cultures of feces provided the best balance between sensitivity (92%) and specificity (83%) compared with the results of culture. Lu et al. (18), who designed novel primers for the detection of *vanA* and *vanB* and who developed a multiplex PCR for the detection of *van* genes directly from EC enrichment broths containing 8 μg of vancomycin per ml, reported a 97.9% sensitivity for VRE detection. Similarly, Palladino et al.

TABLE 2. Characteristics of vancomycin-resistant organisms isolated from five fecal specimens that contained *vanB* genes after anaerobic subculture, but no easily identifiable VRE, by routine methods

Specimen	Isolate	Gram stain result	Identification based on 16S rRNA gene (accession no.)	% Sequence identity
1	MLG856-1	Positive, bacillus	*Clostridium hathewayi* (AJ311620)	98
	MLG856-2	Positive, coccus	*Enterococcus faecium* (AF070223)	99
2	MLG480	Negative,[a] bacillus	*Clostridium innocuum* (AF028352)	91
3	MLG080-1	Variable,[a] bacillus[a]	*Clostridium bolteae* (AJ08452)	98
	MLG080-3	Variable, coccobacillus[b]	*Ruminococcus lactaris* (L76602)	91
4	MLG392	Positive, bacillus	*Clostridium hathewayi* (AJ311620)	98
5	MLG661	Positive, bacillus	*Clostridium hathewayi* (AJ311620)	98

[a] Not all species classified in the genus *Clostridium* stain gram positive, and staining may vary depending on the medium used and the age of the culture (15).
[b] Anaerobic gram-positive cocci may be elongated and resemble coccobacilli and lose their positivity with age (20).

(22) reported a sensitivity of detection of 88% with the primers designed by Dutka-Malen et al. (7) for a *vanA-vanB* multiplex real-time LightCycler PCR with EC enrichment broths containing 8 μg of vancomycin per ml. Notably, however, neither of those studies considered the sensitivity of detection of each genotype separately. In comparison, we found the Dutka-Malen primers to be insensitive (regardless of whether we used an annealing temperature of 54 or 50°C), detecting only 17 to 67% of *vanB* VRE culture-positive specimens, depending on the enrichment broth used.

Interestingly, we found that the two most sensitive methods (those with the Bell and Stinear primers) resulted in a substantial number of false-positive results in which no VRE could be identified. Paule et al. (24) reported similar results when they compared culture with a direct multiplex PCR with the Dutka-Malen *vanA*- and *vanB*-specific primers for the detection of VRE from rectal swabs. Similarly, Sloan et al. (29) reported a higher frequency of discordant results for *vanB* gene detection when they compared a LightCycler *vanA-vanB* detection assay with culture for the direct detection of VRE from perianal stool swab specimens. Such results may be due to the presence of nonviable VRE, viable nonculturable VRE, or a low burden of VRE (17, 24, 29). However, the presence of nonenterococcal reservoirs of *vanB* has been described previously (19, 27, 31). Notably, we isolated five anaerobic gram-positive bacilli related to the clostridia and one *Ruminococcus* sp. from five PCR-positive, VRE culture-negative fecal specimens. These six anaerobes contained a *vanB* ligase gene identical to that identified in *vanB*-containing VRE strains. One of these isolates was obtained from a fecal specimen that also contained low numbers of *vanB E. faecium*. Since approximately 40% of the bacterial community of the bowel has yet to be cultivated in vitro, presumably due to their fastidious growth and nutritional requirements, the identification of other vancomycin-resistant anaerobes remains a possibility (32).

It is important that the majority of our fecal specimens were obtained from patients requiring long-term hemodialysis, so we are uncertain whether factors related to their underlying illness and/or treatment may have influenced the likelihood that these patients were fecally colonized with *vanB*-containing anaerobes. Moreover, as all our patients with VRE were colonized with *vanB*-type VRE, we cannot extrapolate our findings regarding the sensitivity and specificity of each of the PCR primers assessed to other *van* genotypes, particularly *vanA*. However, given the reports of nonenterococcal reservoirs of *vanA*, it will be important to assess the sensitivity and specificity of PCR for the detection of *vanA* in a mixed culture for the identification of *vanA*-type VRE (4, 10, 26). Regardless of these caveats, clinicians and infection control practitioners who rely on molecular analysis of feces to rapidly identify patients who are fecally colonized with VRE need to be aware of the potential role of *vanB* gene-containing anaerobes in causing false-positive interpretations of the presence of VRE.

ACKNOWLEDGMENTS

We thank S. Xie and K. K. Pertile for generous technical assistance.

This study received no financial support, and the authors have no conflict of interest.

REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Bell, J. M., J. C. Paton, and J. Turnidge.** 1998. Emergence of vancomycin-resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. J. Clin. Microbiol. **36:**2187–2190.
3. **Carias, L. L., S. D. Rudin, C. J. Donskey, and L. B. Rice.** 1998. Genetic linkage and cotransfer of a novel, vanB-containing transposon (Tn*5382*) and a low-affinity penicillin-binding protein 5 gene in a clinical vancomycin-resistant *Enterococcus faecium* isolate. J. Bacteriol. **180:**4426–4434.
4. **Centers for Disease Control and Prevention.** 2002. *Staphylococcus aureus* resistant to vancomycin—United States, 2002. Morb. Mortal. Wkly. Rep. **51:**565–567.
5. **D'Agata, E. M. C., 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.
6. **Dahl, K. H., G. S. Simonsen, O. Olsvik, and A. Sundsfjord.** 1999. Heterogeneity in the *vanB* gene cluster of genomically diverse clinical strains of vancomycin-resistant enterococci. Antimicrob. Agents Chemother. **43:**1105–1110.
7. **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. (Erratum, **33:** 1434.
8. **Edberg, S. C., C. J. Hardalo, C. Kontnick, and S. Campbell.** 1994. Rapid detection of vancomycin-resistant enterococci. J. Clin. Microbiol. **32:**2182–2184.
9. **Evers, S., P. E. Reynolds, and P. Courvalin.** 1994. Sequence of the *vanB* and *ddl* genes encoding D-alanine: D-lactate and D-alanine: D-alanine ligases in vancomycin resistant *Enterococcus faecalis* V583. Gene **140:**97–102.
10. **Fontana, R., M. Ligozzi, C. Pedrotti, E. M. Padovani, and G. Cornaglia.** 1997. Vancomycin-resistant *Bacillus circulans* carrying the *vanA* gene responsible for vancomycin-resistance in enterococci. Eur. J. Clin. Microbiol. Infect. Dis. **16:**473–474.
11. **Garnier, F., S. Taourit, P. Glaser, P. Courvalin, and M. Galimand.** 2000. Characterization of transposon Tn*1549*, conferring VanB-type resistance in *Enterococcus* spp. Microbiology **146:**1481–1489.
12. **Grayson, M. L., E. A. Grabsch, P. D. R. Johnson, D. Olden, M. Aberline, H. Y. Li, G. Hogg, M. Abbott, and P. G. Kerr.** 1999. Outcome of a screening program for vancomycin-resistant enterococci in a hospital in Victoria. Med. J. Aust. **171:**133–136.
13. **Gurtler, V., V. A. Wilson, and B. C. Mayall.** 1991. Classification of medically important clostridia using restriction endonuclease site differences of PCR-amplified 16S rDNA. J. Gen. Microbiol. **137:**2673–2679.
14. **Jayaratne, P., and C. Rutherford.** 1999. Detection of clinically relevant genotypes of vancomycin-resistant enterococci in nosocomial surveillance specimens by PCR. J. Clin. Microbiol. **37:**2090–2092.
15. **Johnson, M. J., E. Thatcher, and M. E. Cox.** 1995. Techniques for controlling variability in Gram staining of obligate anaerobes. J. Clin. Microbiol. **33:** 755–758.
16. **Kak, V., and J. W. Chow.** 2002. Acquired antibiotic resistances in enterococci, p. 355–383. *In* D. B. Clewell, P. Courvalin, G. M. Dunny, B. E. Murray, and L. B. Rice (ed.), The enterococci. American Society for Microbiology, Washington, D.C.
17. **Lleò, M. M., B. Bonato, C. Signoretto, and P. Canepari.** 2003. Vancomycin resistance is maintained in enterococci in the viable but nonculturable state and after division is resumed. Antimicrob. Agents Chemother. **47:**1154–1156.
18. **Lu, J. J., C. L. Perng, T. S. Chiueh, S. Y. Lee, C. H. Chen, F. Y. Chang, C. C. Wang, and W. M. Chi.** 2001. Detection and typing of vancomycin-resistance genes of enterococci from clinical and nosocomial surveillance specimens by multiplex PCR. Epidemiol. Infect. **126:**357–363.
19. **Mevius, D., L. Devriese, P. Butaye, P. Vandamme, M. Verschure, and K. Veldman.** 1998. Isolation of glycopeptide resistant *Streptococcus gallolyticus* strains with *vanA*, *vanB*, and both *vanA* and *vanB* genotypes from faecal samples of veal calves in The Netherlands. J. Antimicrob. Chemother. **42:** 275–276.
20. **Moncla, B. J., and S. L. Hillier.** 2003. *Peptostreptococcus*, *Propionibacterium*, *Lactobacillus*, *Actinomyces*, and other non-spore-forming anaerobic gram-positive bacteria, p. 857–879. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
21. **Padiglione, A., R. Wolfe, E. Grabsch, D. Olden, S. Pearson, C. Franklin, D. Spelman, B. Mayall, P. D. R. Johnson, and M. L. Grayson.** 2003. Risk factors for new detection of vancomycin-resistant enterococci in acute-care hospitals that employ strict infection control procedures. Antimicrob. Agents Chemother. **47:**2492–2498.
22. **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.

23. **Patel, R., J. R. Uhl, P. Kohner, M. K. Hopkins, J. M. Steckelberg, B. Kline, and F. R. Cockerill III.** 1998. DNA sequence variation within *vanA*, *vanB*, *vanC-1*, and *vanC-2/3* genes of clinical *Enterococcus* isolates. Antimicrob. Agents Chemother. **42:**202–205.
24. **Paule, S., W. E. Trick, F. C. Tenover, M. Lankford, S. Cunningham, V. Stosor, R. L. Cordell, and L. R. Peterson.** 2003. Comparison of PCR assay to culture for surveillance detection of vancomycin resistant enterococci. J. Clin. Microbiol. **41:**4805–4807.
25. **Petrich, A. K., K. E. Luinstra, D. Groves, M. A. Chernesky, and J. B. Mahony.** 1999. Direct detection of *vanA* and *vanB* genes in clinical specimens for rapid identification of vancomycin resistant enterococci (VRE) using multiplex PCR. Mol. Cell. Probes **13:**275–281.
26. **Power, E. G. M., Y. H. Abdulla, H. G. Talsania, W. Spice, S. Aathithan, and G. L. French.** 1995. *van A* genes in vancomycin-resistant clinical isolates of *Oerskovia turbata* and *Arcanobacterium* (*Corynebacterium*) *haemolyticum*. J. Antimicrob. Chemother. **36:**595–606.
27. **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.
28. **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.
29. **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.
30. **Stinear, T., J. K. Davies, G. A. Jenkin, F. Portaels, B. C. Ross, F. Oppedisano, M. Purcell, J. A. Hayman, and P. D. R. Johnson.** 2000. A simple PCR method for rapid genotype analysis of *Mycobacterium ulcerans*. J. Clin. Microbiol. **38:**1482–1487.
31. **Stinear, T. P., D. C. Olden, P. D. R. Johnson, J. K. Davies, and M. L. Grayson.** 2001. Enterococcal *vanB* resistance locus in anaerobic bacteria in human faeces. Lancet **357:**855–856.
32. **Tannock, G. W., K. Munro, H. J. M. Harmsen, G. W. Welling, J. Smart, and P. K. Gopal.** 2000. Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. Appl. Environ. Microbiol. **66:**2578–2588.
33. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multi-sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.