ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2006, p. 2217–2221 Vol. 50, No. 6
0066-4804/06/$08.00+0 doi:10.1128/AAC.01541-05

VanG-Type Vancomycin-Resistant *Enterococcus faecalis* Strains Isolated in Canada

David A. Boyd,[1] Tim Du,[1] Romeo Hizon,[1] Brynn Kaplen,[1] Travis Murphy,[1] Shaun Tyler,[1] Shirley Brown,[2] Frances Jamieson,[2] Karl Weiss,[3] Michael R. Mulvey,[1]* and the Canadian Nosocomial Infection Surveillance Program

National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada[1]*; Central Public Health Laboratory, Ontario Ministry of Health, Toronto, Ontario, Canada*[2]*; and Hôpital Maisonneuve-Rosemont, Montreal, Quebec, Canada*[3]

Received 1 December 2005/Returned for modification 2 January 2006/Accepted 26 March 2006

***Enterococcus faecalis* G1-0247 (vancomycin MIC, 16 μg/ml) was found to harbor a *vanG* operon 99% identical to the *vanG* operon in *E. faecalis* BM4518. *E. faecalis* N03-0233 (vancomycin MIC, 16 μg/ml) was found to harbor a novel *vanG* operon, *vanG2*, on an element in a different chromosomal location than the *vanG*-harboring elements in G1-0247 and BM4518.**

The *vanG* operon provides low-level resistance to vancomycin through the action of the D-Ala–D-Ser VanG ligase (9). The operon consists of three regulatory genes, $vanU_GR_GS_G$, which are cotranscribed from the P_{UG} promoter, followed by the resistance region consisting of five genes, $vanY_GW_GGXY_GT_G$, which are cotranscribed from the P_{YG} promoter (1). To date the *vanG* operon has been found only in a few related strains of *Enterococcus faecalis* isolated in Australia (1, 7). The transfer of VanG resistance was via ca.-240-kb elements to the chromosome of susceptible *E. faecalis* strains (1). The *vanG* operon and the chromosomal insertion site of the element harboring it have been characterized in *E. faecalis* BM4518 (vancomycin MIC, 16 μg/ml; teicoplanin MIC, 0.5 μg/ml) (1).

This report describes the characterization of two *E. faecalis* strains exhibiting low-level vancomycin resistance that were isolated in Canada.

Genomic libraries were constructed with partial Sau3A fragments using Lambda ZAP (Stratagene, La Jolla, CA). Primers used in PCRs are listed in Table 1. Vectorette PCR, inverse PCR, and thermal asymmetric interlaced (TAIL)-PCR (5, 6, 10) were used to isolate genomic regions that could not be isolated from the genomic libraries.

E. faecalis G1-0247 (vancomycin MIC, 16 μg/ml, and teicoplanin MIC, 0.75 μg/ml by Etest; AB Biodisk, Solna, Sweden) was isolated at Hôpital Maisonneuve-Rosemont, Montreal, Quebec, in December 2001 from a rectal swab from a patient with type 1 diabetes with complications and chronic renal failure. The patient had several hospitalizations since March 2000 and received vancomycin on several occasions during this period. Further analysis of *E. faecalis* G1-0247 by PCR gave a positive reaction for the *vanG* gene. Using a combination of cloning, PCR techniques, and sequencing, we characterized 14,301 bp encompassing the *vanG* operon (accession no. DQ212986) (Fig. 1). Analysis revealed that the *E. faecalis* BM4518 and G1-0247 *vanG* operon regions shared >99% nucleotide identity, with there being only three differences. One was a T at position 1950 (accession no. DQ212986) in the *orfG23-vanU*$_G$ intergenic region, which was a C in BM4518 (position 17015 of accession no. AY271782), and one was a G at position 5930 (accession no. DQ212986) in the first position of codon 265 of the *vanW*$_G$ gene (GAA→Glu-265), which was an A in BM4518 (AAA→Lys-265; position 20994 in accession no. AY271782). The third difference was a T at position 359 of the *E. faecalis* G1-0247 *vanY*$_G$ gene (position 4581 in accession no. DQ212986) which was absent in the BM4518 *vanY*$_G$ gene. Hence, whereas in *E. faecalis* BM4518 the resulting frameshift leads to the introduction of a stop codon 6 bp further downstream (1), G1-0247 contains a full-length *vanY*$_G$ gene. However, *E. faecalis* BM4518 produces another inducible D,D-carboxypeptidase activity presumably located elsewhere in the chromosome, and so $VanY_G$ activity is presumably not essential for resistance (1). Whether the *E. faecalis* G1-0247 $VanY_G$ protein is active and whether another D,D-carboxypeptidase activity is present await biochemical evidence. Downstream of the *vanG* operon were two open reading frames (ORFs) located between *orfG24* and *orfG25* that were not annotated for *E. faecalis* BM4518 (1). The first ORF, *orfG26*, is 429 bp (positions 10874 to 11302 in accession no. DQ212986) and codes for a putative protein of 142 amino acids exhibiting 50% identity to a sigma 24 homolog identified from the *Streptococcus suis* 89/1591 genome (GenBank locus tag Ssui801000309). The second ORF, *orfG27*, is 372 bp (positions 11889 to 12260 of accession no. DQ212986) and codes for a putative protein of 123 amino acids exhibiting 63% identity to the PemK-like MazF protein of *Thermoanaerobacter tengcongensis* MB4 (accession no. AE008691, locus tag TTE2166). Analysis of *orfG25* and downstream flanking DNA showed 100% identity between *E. faecalis* BM4518 and G1-0247 in this region, indicating that an element harboring *vanG* in G1-0247 is inserted in the identical location, near the 3′ end of a gene labeled *orfG1* in BM4518 (Fig. 1). In the fully sequenced *E. faecalis* V583 genome this gene (locus tag EF0728) is located at positions 686280 to 687653 (8). The left junction region of the *E. faecalis*

* Corresponding author. Mailing address: National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St., Winnipeg, Manitoba, Canada R3E 3R2. Phone: (204) 789-2133. Fax: (204) 789-5020. E-mail: Michael_mulvey@phac-aspc.gc.ca.

TABLE 1. Primers used in this study

Name	Sequence (5′–3′)	Coordinates	Technique	Reference
vanG1	CGGTTGTGCCGTACTTGGC	21729–21745[a]	PCR	7
vanG2	GGGTAAAGCCATAGTCTGGGGC	22518–22539[a]	PCR	7
T_G-DN1	TATGTGCTGATTGGTGGACG	24757–24776[a]	PCR	This study
orfG25-UP1	TTAGAACGGCCACCACCTTGT	29345–29365[a]	PCR	This study
trmA-1	TTGAGAATGCCACTTTCACC	687343–687362[b]	TAIL-PCR	This study
trmA-2	AACCAGATGTTGTGGTGGTG	687402–687431[b]	TAIL-PCR	This study
trmA-3	AGGGTTAGACGGTCAATTAG	687446–687465[b]	TAIL-PCR	This study
S_{G2}-UP1	TAATAAAGACCCAACCTATC	2165–2184[c]	Vectorette PCR	This study
Mwo-F	AGTATCTTCGACCTTTGCAGTCAGC	1480–1504[c]	Inverse PCR	This study
Mwo-R	ACGCTATAATCAGCCCATAAAC	1609–1630[c]	Inverse PCR	This study
MF2	TCTGTACCATTATAAAATTT	1337–1354[c]	TAIL-PCR	This study
MF3	ATTTGCACTCCACATTAACC	1224–1243[c]	TAIL-PCR	This study
dctP-DN3	AGCAGTTATGGTATTGGCAG	399411–399430[b]	TAIL-PCR	This study
dctP-DN2	GTCAGTGGATCAGCATTAG	399670–399688[b]	TAIL-PCR	This study
dctP-DN1	CATCTCCAGATGATTTGAAAGG	399881–399902[b]	TAIL-PCR	This study
AD4	STTGNTASTNCTNTGC	N/A[d]	TAIL-PCR	10
AD5	NTCGASTWTSGWGTT	N/A[d]	TAIL-PCR	10
AD6	WGTGNAGWANCANAGA	N/A[d]	TAIL-PCR	10
V1	GGIGARGAYGGIYSITTICARGG	N/A[d]	D-Ala:D-Xxx ligases	3
V2	TGRAAICCIGGIADIGTRTT	N/A[d]	D-Ala:D-Xxx ligases	3

[a] From *E. faecalis* BM4518 *vanG* operon, accession no. AY271782.
[b] From *E. faecalis* V583 genome sequence, accession no. AE016830.
[c] From *E. faecalis* N03-0233 *vanG2* operon, accession no. DQ222944.
[d] N is any base; I is inosine; R is A or G; S is G or C; W is A or T; Y is C or T.

G1-0247 *vanG* element (accession no. DQ212987) was isolated by TAIL-PCR, and analysis showed that, whereas the locus EF0728 sequences were 100% identical, the next 343 bp, which define the 3′ end of *orfG1*, exhibited only 86% identity, including differences leading to five amino acid changes (data not shown). Further, after the *orfG1* stop codon the sequences diverged even more significantly, exhibiting only 43% identity. In the five VanG-type clinical isolates studied, the element harboring the resistance genes was located on chromosomal SmaI fragments sized from 400 to 530 kb (1). It was postulated that each strain acquired different elements independently or that the same transconjugant underwent DNA rearrangements upon, or following, integration. The differences in *vanG* elements may occur near the left end, whereas the right end containing the vancomycin resistance genes and the putative site-specific OrfG25 recombinase is conserved.

E. faecalis N03-0233 (vancomycin MIC, 16 μg/ml; teicoplanin MIC, 0.5 μg/ml), isolated in 2003 from a rectal swab of a 55-year-old patient hospitalized in southern Ontario, was negative by PCR for *vanA*, *vanB*, *vanD*, *vanE*, and *vanG*. Analysis of a 600-bp product obtained with the V1/V2 degenerate primer pair for D-Ala–D-Xxx ligase genes (3) revealed a partial gene, labeled *vanG2*, with 87% nucleotide identity to the *vanG* gene from *E. faecalis* BM4518. Using a combination of cloning, PCR, and sequencing, a total of 12,547 bp of the region containing the *vanG2* operon was determined (accession no. DQ222944) (Fig. 1). The *vanG2* operon was found to consist of seven ORFs, the regulatory region consisting of $vanU_{G2}R_{G2}S_{G2}$, and the resistance region consisting of $vanW_{G2}GXY_{G2}T_{G2}$. Analysis revealed that the failure of the vanG1/vanG2 primer pair to amplify the *vanG2* gene was due to sequence differences in the primer binding sites (data not shown). There was no $vanY_{G2}$ gene found between $vanS_{G2}$ and $vanW_{G2}$. The proteins encoded by the *vanG2* operon share between 74 and 96% identity to the corresponding proteins encoded by the *vanG* operon (Fig. 1). The region downstream of $vanT_{G2}$ is similar to the corresponding region in the *vanG* operon, though the OrfG25 and $OrfG_2 25$ proteins exhibit only 21% identity. There was no $orfG_2 23$ homolog present in the *vanG2* operon upstream of $vanU_{G2}$. Analysis downstream of $orfG_2 25$ showed that this region was 100% identical to the *dctP* gene of *E. faecalis* V583, which encodes the TRAP dicarboxylate transporter DctP subunit (locus tag EF0429) (8). Thus, the element containing the *vanG2* operon was found in a different chromosomal location than the element harboring the *vanG* operon. The left junction of the *vanG2* element was isolated by TAIL-PCR (accession no. DQ222945). Analysis revealed the target site of integration was the 2-bp sequence CA equivalent to positions 400063 and 400064 of the *E. faecalis* V583 genome (8). In *E. faecalis* N03-0233 this 2-bp sequence flanks imperfect 8-bp inverted repeats that define the ends of the *vanG2* element (left end, positions 142 to 149 of accession no. DQ222945; right end, positions 12210 to 12217 of accession no. DQ222944) (Fig. 1). We detected a 300-bp ORF, labeled $orfG_2 1$, starting 155 bp downstream of the left junction integration site, whose putative product showed no significant homology to extant proteins in the GenBank database.

Vancomycin resistance was inducible in both *E. faecalis* G1-0247 and N03-0233 (data not shown) as it was in BM4518 (1). Given the near identity of the *vanG* operons, regulation of resistance in *E. faecalis* G1-0247 is likely the same as in BM4518. An alignment of the regions upstream of $vanU_{G2}$ and $vanW_{G2}$ with the P_{UG} and P_{YG} promoters indicated that similar promoter regions exist in the *vanG2* operon (P_{UG2} and P_{WG2} in Fig. 2A and B, respectively). Further, putative $VanR_G$/P-$VanR_G$ binding regions are conserved upstream of the −35 box in the P_{WG2} region (Fig. 2B and C). It was postulated for the *E. faecalis* BM4518 *vanG* operon that one or more of these 12-bp regions are the core binding sites for $VanR_G$/P-$VanR_G$, allowing for inducible positive control of the resistance genes

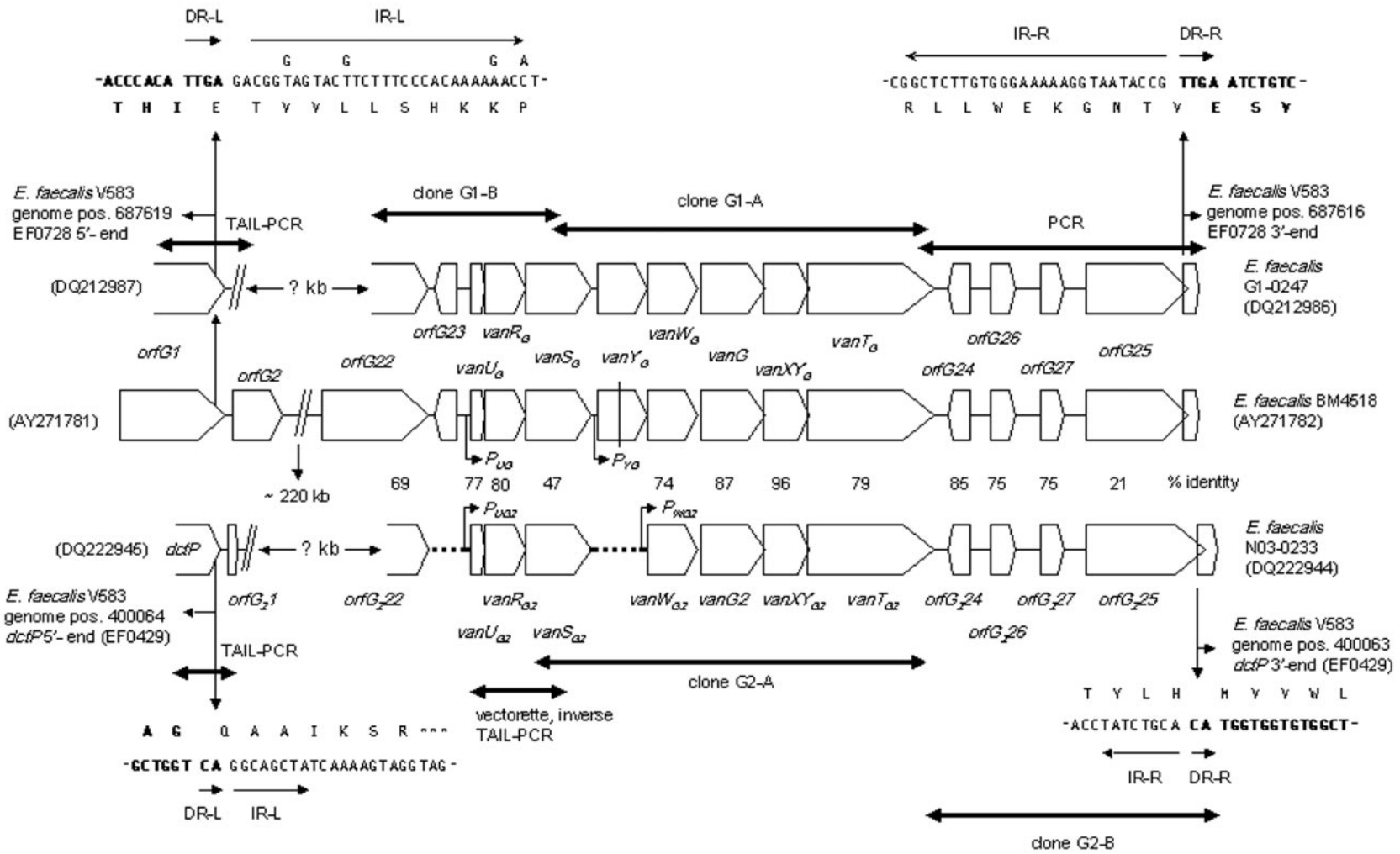

FIG. 1. Schematic of left junction regions and right junction regions from the *vanG* and *vanG2* elements from *E. faecalis* G1-0247 and N03-0233, respectively, compared to the corresponding regions from *E. faecalis* BM4518. The regions cloned or characterized by PCR are indicated by two-headed arrows. Regions missing in the *vanG2* operon compared to the corresponding regions in the *vanG* operon are indicated by dashed lines. Percent identities between the corresponding proteins encoded by the *vanG* and *vanG2* regions are shown. The sequences surrounding the left and right genomic junctions of the *vanG* and *vanG2* elements are shown at the bottom; nucleotides shown above the *E. faecalis* G1-0247 IR-L indicate differences from the BM4518 sequence. DR-L, direct repeat left; DR-R, direct repeat right; IR-L, inverted repeat left; IR-R, inverted repeat right.

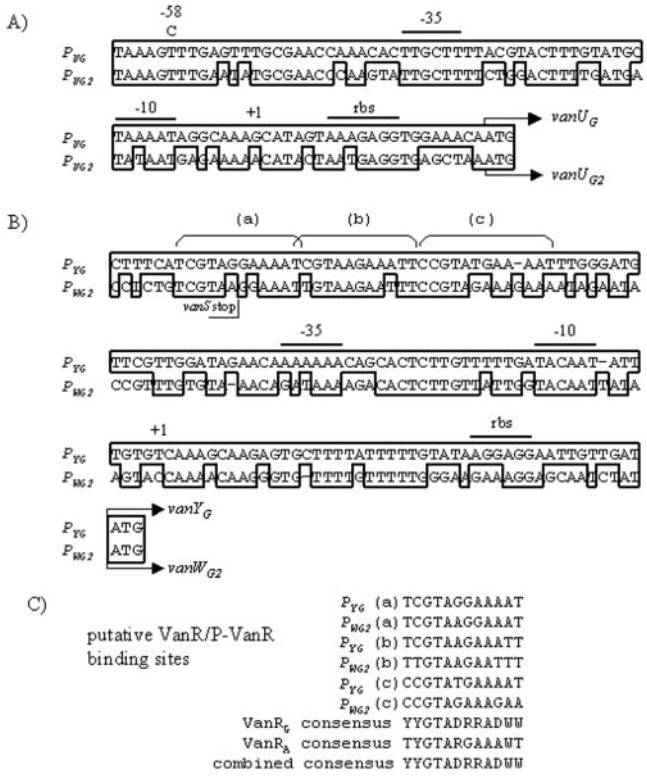

FIG. 2. Alignments of the promoter regions from the *vanG* and *vanG2* operons. Identical nucleotides are blocked. A) Alignment of the regulatory gene promoters P_{UG} and P_{UG2}; B) alignment of the resistance gene promoters P_{YG} and P_{WG2}; C) alignment of the putative binding sites for $VanR_G$/P-$VanR_G$ and $VanR_{G2}$/P-$VanR_{G2}$ (a, b, and c in panel B) and the consensus binding site for $VanR_A$/P-$VanR_A$ (4). An overall consensus binding site is shown. D is A, G, or T; R is A or G; W is A or T; and Y is C or T.

(1). It was noted (1) that these regions are highly conserved relative to the $VanR_A$/P-$VanR_A$ consensus binding region in VanA-type *Enterococcus faecium* (Fig. 2C) (4).

In *E. faecalis* BM4518, vancomycin resistance was cotransferred with *ermB* at a low frequency (2×10^{-9} copies per donor) but only with selection on erythromycin (1). Both *E. faecalis* G1-0247 and N03-0233 harbored *ermB*, and transconjugants of *E. faecalis* JH2-2 carrying *ermB* could easily be selected on erythromycin after mating with either strain by cross-streaking on solid media. However, analysis of six transconjugants of each type showed that none harbored either *vanG* or *vanG2*. As with BM4518, no transconjugants could be selected on vancomycin. As we only analyzed a total of 12 transconjugants, it may be that *ermB vanG* transconjugants did exist but weren't characterized. Alternatively, *ermB* may be found on a mobile genetic element either separate from or embedded within the elements harboring *vanG* or *vanG2* and more readily transferred. Also, the functions necessary for *vanG* transfer may not be functional in *E. faecalis* G1-0247 or N03-0233, or the conditions necessary for transfer were not met with the mating method used.

Enterococci harboring *vanG*-type genes are extremely rare; to date only a few strains from Australia and Canada have been isolated. In a previous study, *vanG* genes exhibiting 77% to 100% identity to the *E. faecalis* BM4518 *vanG* gene have been found in the fecal flora of humans, though the organisms harboring these genes could not be identified (2). The similarity of the regions characterized here and in *E. faecalis* BM4518 indicate that the *vanG* and *vanG2* operons reside on elements that belong to a family of putatively conjugative related genetic elements. The difference in the chromosomal locations of the elements is most likely due to the action of the diverse OrfG25 site-specific recombinases. The absence of a $vanY_{G2}$ gene in the *vanG2* operon is likely not detrimental to resistance expression, as the $VanY_G$ activity may be redundant in enterococci if the strain contains another D,D-carboxypeptidase activity, as found for *E. faecalis* BM4518 (1).

Nucleotide sequence accession numbers. The *vanG* operon region and left junction region of the *vanG* element from *E. faecalis* G1-0247 were assigned accession no. DQ212986 and DQ212987, respectively, and the *vanG2* operon region and left junction region of the *vanG2* element from *E. faecalis* N03-0233 were assigned accession no. DQ222944 and DQ222945, respectively, in the GenBank database.

We thank Patrice Courvalin (Institut Pasteur) for providing us with sequence data before its publication.

Members of the Canadian Nosocomial Infection Surveillance Program are as follows: Elizabeth Bryce, Vancouver General Hospital, Vancouver, BC; John Conly, Foothills Medical Centre, Calgary, Alta.; Gordon Dow, The Moncton Hospital, Moncton, NB; John Embil, Health Sciences Centre, Winnipeg, Man.; Joanne Embree, Health Sciences Centre, Winnipeg, Man.; Michael Gardam, University Health Network, Toronto, Ont.; Denise Gravel, Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada; Elizabeth Henderson, Peter Lougheed Centre, Calgary, Alta.; James Hutchinson, Health Sciences Centre, St. John's, Nfld.; Michael John, London Health Sciences Centre, London, Ont.; Lynn Johnston, Queen Elizabeth II Health Sciences Centre, Halifax, NS; Pamela Kibsey, Victoria General Hospital, Victoria, BC; Joanne Langley, I. W. K. Grace Health Science Centre, Halifax, NS; Mark Loeb, Hamilton Health Sciences Corporation, Hamilton, Ont.; Anne Matlow, Hospital for Sick Children, Toronto, Ont.; Allison McGeer, Mount Sinai Hospital, Toronto, Ont.; Sophie Michaud, CHUS-Hôpital Fleurimont, Sherbrooke, Que.; Mark Miller, SMBD-Jewish General Hospital, Montreal, Que.; Dorothy Moore, Montreal Children's Hospital, Montreal, Que.; Michael Mulvey, National Microbiology Laboratory, Public Health Agency of Canada; Marianna Ofner-Agostini, Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada; Shirley Paton, Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada; Virginia Roth, The Ottawa Hospital, Ottawa, Ont.; Andrew Simor, Sunnybrook and Women's College Health Sciences Centre, Toronto, Ont.; Geoffrey Taylor, University of Alberta Hospital, Edmonton, Alta.; Monali Varia, Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada; Mary Vearncombe, Sunnybrook and Women's College Health Sciences Centre, Toronto, Ont.; Alice Wong, Royal University Hospital, Saskatoon, Sask.; and Dick Zoutman, Kingston General Hospital, Kingston, Ont.

REFERENCES

1. **Depardieu, F., M. G. Bonora, P. E. Reynolds, and P. Courvalin.** 2003. The *vanG* glycopeptide resistance operon from *Enterococcus faecalis* revisited. Mol. Microbiol. **50:**931–948.
2. **Domingo, M.-C., A. Huletsky, R. Giroux, K. Boissinot, F. J. Picard, P. Lebel, M. J. Ferraro, and M. G. Bergeron.** 2005. High prevalence of glycopeptide resistance genes *vanB*, *vanD*, and *vanG* not associated with enterococci in human fecal flora. Antimicrob. Agents Chemother. **49:**4784–4786.
3. **Dukta-Malen, S., C. Molinas, M. Arthur, and P. Courvalin.** 1992. Sequence of the *vanC* gene of *Enterococcus gallinarum* BM4174 encoding a D-alanine: D-alanine ligase-related protein necessary for vancomycin resistance. Gene **112:**53–58.
4. **Holman, T. R., Z. Wu, B. L. Wanner, and C. T. Walsh.** 1994. Identification

of the DNA-binding site for the phosphorylated VanR protein required for vancomycin resistance in *Enterococcus faecium*. Biochemistry **33:**4625–4631.
5. **Liu, Y.-G., N. Mitsukawa, T. Oosumi, and R. F. Whittier.** 1995. Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J. **8:**457–463.
6. **Liu, Y.-G., and R. F. Whittier.** 1995. Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. Genomics **25:**674–681.
7. **McKessar, S. J., A. M. Berry, J. M. Bell, J. D. Turnidge, and J. C. Paton.** 2000. Genetic characterization of *vanG*, a novel vancomycin resistance locus of *Enterococcus faecalis*. Antimicrob. Agents Chemother. **44:**3224–3228.
8. **Paulsen, I. T., L. Banerjei, G. S. A. Myers, K. E. Nelson, R. Seshadri, T. D. Read, D. E. Fouts, J. A. Eisen, S. R. Gill, J. F. Heidelberg, H. Tettelin, R. J. Dodson, L. Umayam, L. Brinkac, M. Beanan, S. Daugherty, R. T. DeBoy, S. Durkin, J. Kolonay, R. Madupu, W. Nelson, J. Vamathevan, B. Tran, J. Upton, T. Hansen, J. Shetty, H. Khouri, T. Utterback, D. Radune, K. A. Ketchum, B. A. Dougherty, and C. M. Fraser.** 2003. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. Science **299:** 2071–2074.
9. **Reynolds, P. E., and P. Courvalin.** 2005. Vancomycin resistance in enterococci due to synthesis of precursors terminating in D-alanyl-D-serine. Antimicrob. Agents Chemother. **49:**21–25.
10. **Singer, T., and E. Burke.** 2003. High-throughput TAIL-PCR as a tool to identify DNA flanking insertions. Methods Mol. Biol. **236:**241–271.