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Molecular Diversity and Evolutionary Relationships of Tn*1546*-Like Elements in Enterococci from Humans and Animals

ROB J. L. WILLEMS,[1]* JANETTA TOP,[1] NICOLE VAN DEN BRAAK,[2] ALEX VAN BELKUM,[2] DIK J. MEVIUS,[3] GIEL HENDRIKS,[1] MARGA VAN SANTEN-VERHEUVEL,[1] AND JAN D. A. VAN EMBDEN[1]

Research Laboratory for Infectious Diseases, National Institute of Public Health and the Environment, 3720 BA Bilthoven,[1] Department of Medical Microbiology & Infectious Diseases, Erasmus Medical Center Rotterdam, 3015 GD Rotterdam,[2] and Department of Bacteriology, DLO-Institute for Animal Science and Health, 8200 AB Lelystad,[3] The Netherlands

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We report on a detailed study on the molecular diversity and evolutionary relationships of Tn*1546*-like elements in vancomycin-resistant enterococci (VRE) from humans and animals. Restriction fragment length polymorphism (RFLP) analysis of the VanA transposon of 97 VRE revealed seven different Tn*1546* types. Subsequent sequencing of the complete VanA transposons of 13 VRE isolates representing the seven RFLP types followed by sequencing of the identified polymorphic regions in 84 other VanA transposons resulted in the identification of 22 different Tn*1546* derivatives. Differences between the Tn*1546* types included point mutations in *orf1*, *vanS*, *vanA*, *vanX*, and *vanY*. Moreover, insertions of an IS*1216V*-IS*3*-like element in *orf1*, of IS*1251* in the *vanS-vanH* intergenic region, and of IS*1216V* in the *vanX-vanY* intergenic region were found. The presence of insertion sequence elements was often associated with deletions in Tn*1546*. Identical Tn*1546* types were found among isolates from humans and farm animals in The Netherlands, suggesting the sharing of a common vancomycin resistance gene pool. Application of the genetic analysis of Tn*1546* to VRE isolates causing infections in hospitals in Oxford, United Kingdom, and Chicago, Ill., suggested the possibility of the horizontal transmission of the vancomycin resistance transposon. The genetic diversity in Tn*1546* combined with epidemiological data suggest that the DNA polymorphism among Tn*1546* variants can successfully be exploited for the tracing of the routes of transmission of vancomycin resistance genes.

In recent years, the nosocomial prevalence of infections caused by vancomycin-resistant enterococci (VRE) has increased significantly in the United States (13, 36), while virtually no VRE have been found in the gut flora of healthy people (16). The epidemiology of VRE in Europe differs from that in the United States. The prevalence of VRE in Europe is low among strains causing hospital-associated infections (20, 22, 44), while VanA-positive enterococci can easily be detected outside the hospital in several European countries (20, 43, 46, 47, 48, 49, 51). A possible source of VRE is the food chain since VRE have been isolated from farm animals and animal products in several European countries (2, 7, 8, 14, 17, 33, 48, 49, 50, 53). It has been suggested that the use of the antibiotic avoparcin as a feed additive in animal husbandry in numerous European countries has resulted in the selection of vancomycin resistance in strains from farm animals (1, 7, 32). This is consistent with the lack of non-hospital-associated VRE in the United States, where the use of avoparcin has not been permitted (16).

Although resistance to glycopeptides has spread primarily in enterococci, *vanA*- and *vanB*-related genes were recently isolated from various other gram-positive bacteria like *Arcanobacterium haemolyticum* (41), *Oerskovia turbata* (41), *Streptococcus bovis* (42), and *Bacillus circulans* (21). Vancomycin resistance may disseminate to other pathogens, such as methicillin-resistant *Staphylococcus aureus* strains, which would result in a highly dangerous pathogen that could cause an infection that would be difficult to treat with currently available antibiotics. Indeed, conjugative transfer of glycopeptide resistance from *Enterococcus faecalis* to *S. aureus* has been reported under laboratory conditions (39). The possibility that such a transfer will eventually occur in nature stresses the need to limit the spread of VRE and to gain insight into the factors that contribute to the selection of VRE and the routes of dissemination.

The genes encoding the VanA and VanB types of vancomycin resistance are located on mobile DNA elements. Therefore, the horizontal transfer of resistance genes among enterococci may have a more significant impact on the dissemination of vancomycin resistance than the clonal spread of resistant enterococci. The isolation of genetically unrelated VREs during well-documented nosocomial outbreaks suggests such a mechanism (10, 15, 24, 35, 38). Thus, direct comparison of the vancomycin resistance determinants may provide additional insight into the epidemiology of vancomycin resistance. The *vanA* gene is the most frequently encountered gene among isolates causing VRE infections in humans (9, 19, 20, 22, 31). This gene is part of the transposable element Tn*1546*, which was first characterized in 1993 by Arthur et al. (5). Genetic heterogeneity in Tn*1546*-related elements has been documented previously (3, 5, 23, 27, 29, 34, 37, 48, 50, 54, 55). The polymorphisms described so far have included insertion of the insertion sequence (IS) elements IS*1216V*, IS*1251*, IS*1476*, and IS*1542* and deletions at both the left (*orf1* side) and right (*vanZ* side) ends of the transposon that includes the *orf1* and *vanZ* genes. Recently, a point mutation in the *vanX* gene has been described (29, 48).

* Corresponding author. Mailing address: Research Laboratory for Infectious Diseases (LIO), National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Phone: 31.30.2744050. Fax: 31.30.2744449. E-mail: rob.willems@rivm.nl.

TABLE 1. Enterococcal isolates used in this study

Strain no.	Strain	Enterococcal species	Source	Country[a]	Tn*1546*-types	Reference
1	9600188	*E. faecium*	Human stool	NL	A2	This study
2	9600205	*E. faecalis*	Human stool	NL	A1	This study
3	9600220	*E. faecium*	Human stool	NL	A1[b]	This study
4	9600224	*E. faecium*	Human stool	NL	A2	This study
5	9600253	*E. faecium*	Human stool	NL	A2	This study
6	9600266	*E. faecium*	Human stool	NL	A2	This study
7	9600276	*E. faecium*	Human stool	NL	A1	This study
8	9600291	*E. faecium*	Human stool	NL	A1	This study
9	9700196	*E. faecium*	Human stool	NL	A1	This study
10	9700228	*E. faecium*	Human stool	NL	A2	This study
11	22-R	*E. faecium*	Human stool	NL	A2	20
12	10-A	*E. faecium*	Human wound	NL	A1	20
13	10-B	*E. faecium*	Human ascites	NL	A2	20
14	10-C	*E. faecium*	Human blood	NL	A2	20
15	10-D	*E. faecium*	Human urine	NL	A2	20
16	10-G	*E. faecium*	Human bile	NL	A2	20
17	10-H	*E. faecium*	Human blood	NL	A2[b]	20
18	10-J	*E. faecalis*	Human ascites	NL	A1	20
19	1245964	*E. faecium*	Human urine	NL	A2	This study
20	2074639	*E. faecium*	Human ascites	NL	A2	This study
21	4252948	*E. faecium*	Human ascites	NL	E6	This study
22	1-A2	*E. gallinarum*	Veal calf	NL	A1[b]	This study
23	1-A6	*E. flavescens*	Veal calf	NL	A3[b]	This study
24	1-A8	*E. faecalis*	Veal calf	NL	B1[b]	This study
25	1-A10	*E. avium*	Veal calf	NL	A1	This study
26	1-A11	*E. faecium*	Veal calf	NL	A4[b]	This study
27	A2	*E. faecium*	Pig	NL	A2	49
28	A4	*E. faecium*	Pig	NL	A2	49
29	A16	*E. faecium*	Pig	NL	A2	49
30	B9	*E. faecium*	Pig	NL	A2	49
31	B37	*E. faecium*	Pig	NL	A2	49
32	M4	*E. faecium*	Pig	NL	A2	49
33	M7	*E. faecium*	Pig	NL	A2	49
34	M11	*E. faecium*	Pig	NL	A2	49
35	O12	*E. faecium*	Pig	NL	A2	49
36	O118	*E. faecium*	Pig	NL	A2	49
37	O122	*E. faecium*	Pig	NL	A2	49
38	chicken 2	*E. faecium*	Chicken	NL	E3	50
39	chicken 3	*E. faecium*	Chicken	NL	A1	50
40	chicken 43	*E. faecium*	Chicken	NL	A2	50
41	chicken 48	*E. faecium*	Chicken	NL	B2	50
42	chicken 57	*E. faecium*	Chicken	NL	E2[b]	50
43	chicken 59	*E. faecium*	Chicken	NL	A1	50
44	chicken 69	*E. faecium*	Chicken	NL	E5	50
45	chicken 72	*E. faecium*	Chicken	NL	A1	50
46	58538 (GP)	*E. faecium*	Human stool	UK	E2	31
47	61741 (GP3)	*E. faecium*	Human stool	UK	A1	8
48	55859 (patient 12)	*E. faecium*	Human stool	UK	D1[b]	31
49	59479	*E. faecium*	Human stool	UK	D1	31
50	60761	*E. faecium*	Human stool	UK	D1	31
51	63910	*E. faecium*	Human stool	UK	C[b]	31
52	67668	*E. faecium*	Human stool	UK	A1	31
53	53864 (patient 3)	*E. faecium*	Human stool	UK	D1	31
54	77364 (patient 10)	*E. faecium*	Human stool	UK	D1	31
55	58155 (patient 9)	*E. faecium*	Human urine	UK	D1	31
56	62899 (patient 11)	*E. faecium*	Human urine	UK	D2	31
57	68521 (patient 15)	*E. faecium*	Human urine	UK	D1	31
58	72801 (patient 12)	*E. faecium*	Human wound	UK	D1	31
59	80103 (BC20)	*E. faecium*	Human blood	UK	D1	8
60	89407 (U22)	*E. faecium*	Human urine	UK	D1	8
61	26712 (patient 1)	*E. faecium*	Human urine	UK	D1	31
62	38658 (patient 2)	*E. faecium*	Human blood	UK	D1	31
63	42757 (patient 3)	*E. faecium*	Human urine	UK	D1	31
64	43088 (patient 4)	*E. faecium*	Human urine	UK	D1	31
65	68140 (patient 10)	*E. faecium*	Human urine	UK	D1	31
66	66925 (patient 13)	*E. faecium*	Human urine	UK	D1	31
67	74198 (patient 14)	*E. faecium*	Human pus	UK	D4	31
68	70040 (patient 16)	*E. faecium*	Human urine	UK	D4	31

Continued on following page

TABLE 1—*Continued*

Strain no.	Strain	Enterococcal species	Source	Country[a]	Tn*1546*-types	Reference
69	75436 (patient 18)	*E. faecium*	Human pus	UK	D1	31
70	S1 (C2)	*E. faecium*	Sewage inlet A	UK	E1[b]	8
71	S5 (L#3)	*E. faecium*	Sewage inlet B	UK	D3	8
72	S10 (C1)	*E. faecium*	Sewage inlet A	UK	E7	8
73	S17 (M7)	*E. faecium*	Sewage inlet B	UK	E3	8
74	S25 (M2)	*E. faecium*	Sewage inlet C	UK	G[b]	8
75	S26 (M3)	*E. faecium*	Sewage inlet C	UK	A2	8
76	S27 (Mixed 0.1#1)	*E. faecium*	Sewage inlet A	UK	A1	8
77	A1 (VF1)	*E. faecium*	Pig	UK	A2	8
78	A6 (Pig 22)	*E. faecium*	Pig	UK	A2	8
79	A10 (Pig 2,19)	*E. faecium*	Pig	UK	A2[b]	8
80	C2 (Sim Chick)	*E. faecium*	Uncooked chicken	UK	B3	8
81	C3 (T2)	*E. faecium*	Uncooked chicken	UK	A1	8
82	C4 (Chicken 1)	*E. faecium*	Uncooked chicken	UK	B1	8
83	C5 (Grade A)	*E. faecium*	Uncooked chicken	UK	E4	8
84	C12 (VF4)	*E. faecium*	Turkey	UK	A1	8
85	C13 (VF7 alfa)	*E. faecium*	Duck	UK	A1	8
86	C14 (VF8)	*E. faecium*	Chicken	UK	A1	8
87	C15 (VF9)	*E. faecium*	Pony	UK	A1	8
88	VS1	*E. faecium*	Human	USA	F2[b]	12
89	VS2	*E. faecium*	Human	USA	F2	12
90	VS3	*E. faecium*	Human	USA	F2	12
91	VS4	*E. faecium*	Human	USA	F1	12
92	VS5	*E. faecium*	Human	USA	F2	12
93	VS6	*E. faecium*	Human	USA	F2	12
94	VS7	*E. faecium*	Human	USA	F2	12
95	VS8	*E. faecium*	Human	USA	F2	12
96	VS9	*E. faecium*	Human	USA	F2	12
97	VS10	*E. faecium*	Human	USA	F2	12

[a] NL, The Netherlands; UK, United Kingdom; USA, United States.
[b] Tn*1546* types which were sequenced entirely.

The aim of the present study was to perform a detailed molecular characterization of the DNA polymorphisms in the VanA gene cluster originating from human and animal sources. By means of restriction fragment length polymorphism (RFLP) analysis and DNA sequencing, 22 different VanA transposon types among 97 VRE strains were identified. Differences included point mutations in the *orf1*, *vanA*, *vanX*, and *vanY* genes, the presence of the IS elements IS*1251* and IS*1216V*, and deletions associated with IS insertions. Indistinguishable Tn*1546*-like elements were found among enterococci isolated from human and animal sources, suggesting the existence of a common vancomycin resistance gene pool.

MATERIALS AND METHODS

Bacterial strains. The VRE used in this study are listed in Table 1. Stool samples from nonhospitalized individuals were collected and cultured in kanamycin-esculin azide enrichment broth (Oxoid Ltd., Basingstoke, United Kingdom) supplemented with 6 μg of vancomycin per ml. Bacteria from tubes whose contents turned black after 1 or 2 days of incubation at 37°C were subcultured onto Slanetz and Bartley agar (Oxoid Ltd.) supplemented with 6 μg of vancomycin per ml. VRE were identified to the species level and were tested for the presence of the *vanA* gene by means of a PCR described by Dutka-Malen et al. (18). Fecal samples from veal calves were examined as described above. Dutch clinical isolates (isolates 11 to 21), pig isolates (isolates 27 to 37), and chicken isolates (isolates 38 to 45) have been described previously (20, 49, 50), as have the isolates from the United Kingdom (isolates 46 to 87) (8, 31) and the United States (isolates 88 to 97) (11, 12).

Susceptibility testing. MICs were determined by the agar dilution method on Mueller-Hinton II agar plates (BBL, Becton Dickinson, Cockeysville, Md.). Inocula (approximately 10^8 CFU/ml) were prepared from overnight cultures on Columbia agar plates supplemented with defribrinated horse blood (Oxoid Ltd.). The antimicrobial agents tested were vancomycin (Eli Lilly, Indianapolis, Ind.), teicoplanin (Hoechst Marion Roussel Inc., Frankfurt, Germany), and avoparcin (Roche Pharmaceuticals, Basel, Switzerland).

PFGE. Pulsed-field gel electrophoresis (PFGE) analysis was performed as described previously (50). The banding patterns were interpreted as described by Tenover et al. (45), and the different types were identified by capital-letter codes.

RFLP analysis. Genomic DNAs from all VRE were isolated by a modification of the initial steps of the method described by Ausubel et al. (6). The bacterial pellets were suspended in 557 μl of 10 mM Tris–1 mM EDTA, and 10 μl of a 50-mg/ml solution of egg white lysozyme (Boehringer Mannheim, Mannheim, Germany) was added. After incubation for 15 min at 37°C, the bacteria were lysed by the addition of 30 μl of 10% sodium dodecyl sulfate and 3 μl of a 20-mg/ml proteinase K (Merck, Darmstadt, Germany) solution. Subsequently, the protocol described by Ausubel et al. (6) was used. Chromosomal DNA preparations were digested with *Hae*III and *Xba*I (Boehringer Mannheim), respectively, separated by agarose gel electrophoresis (1.5% agarose gels), transferred onto a Hybond N^+ nylon membrane (Nycomed Amersham plc, Buckinghamshire, United Kingdom) with a vacuum blotting system (Millipore, Bedford, Mass.), and subsequently hybridized with internal Tn*1546* PCR fragments (probes 1, 2, 3, and 4 generated with primers 22.F-1913.R, 3514.F-5374.R, 5235.F-7035.R, and 8544.F-10716.R, respectively; see Table 2 and Fig. 1). Labeling of the PCR fragments and subsequent detection of hybrids were performed as described in the instructions for the ECL direct nucleic acid labeling and detection kit (Nycomed Amersham plc.).

DNA sequence analysis. The PCR products described below were purified with a Qiagen PCR purification kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. Subsequently, the purified PCR products were sequenced directly with the ABI PRISM Big Dye cycle sequencing ready reaction kit (Perkin-Elmer, Applied Biosystems, Foster City, Calif.) on an ABI PRISM 377 DNA Sequencer (Perkin-Elmer). All VRE isolates were analyzed for the point mutation in the *orf1*, *vanS*, and *vanX* genes. To determine the DNA sequence of the left end of the truncated VanA transposon derivatives, type A2, B3, C, D1 to D4, E1 to E7, F1, F2, and G DNA fragments were amplified with Tn*1546* primer 184.R, 1009.R, 1292.R, or 4511R in combination with IS*1216* primer IS1216V.B. The exact integration site and orientation of IS*1216V* in the *vanX-vanY* intergenic region were determined by amplifying a DNA fragment with primers 7875.F and 10716.R, and the sequence was determined with the IS*1216V* primers IS1216.E and IS1216.F. Finally, all VRE isolates carrying Tn*1546* types F1 and F2 were analyzed for the mutation in the *vanA* and *vanY* genes, as determined with isolate VS1, by sequencing the corresponding region

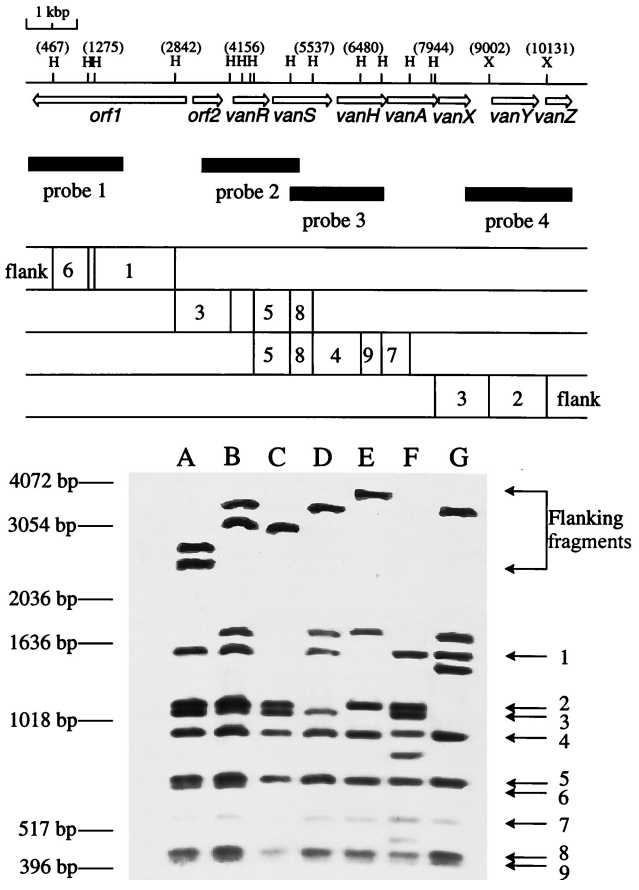

FIG. 1. RFLP analysis and physical and genetic maps of Tn*1546*. The position and direction of transcription of genes and open reading frames (orf's) are indicated with open arrows. Black horizontal bars indicate the position of internal Tn*1546* fragments used as probes (probes 1 to 4). The numbers 1 to 9 represent the restriction fragments visualized after hybridization with the Tn*1546*-specific probes 1 to 4 and are indicated on the right side of the blot. The positions of the molecular size markers are indicated on the left side of the blot. Letters above the lanes represent the Tn*1546* RFLP types. Only the restriction enzyme recognition sites relevant for this study are shown. H, *Hae*III; X, *Xba*I. The positions of some restriction sites are indicated in parentheses.

of the PCR fragments generated with primers 6964.F, 8691.R, 7875.F, and 10716.R.

RESULTS

RFLP analysis of Tn*1546*-like elements. In order to identify polymorphic regions in the vancomycin-resistant transposon Tn*1546*, 97 different *vanA* gene-carrying VRE (Table 1) isolated from different sources were analyzed by means of RFLP analysis.

Seven different RFLP patterns, types A to G, were detected (Fig. 1). The banding pattern of type A was identical to the predicted pattern for the published sequence of Tn*1546* (5). For types B, D, E, and G, an additional fragment of approximately 1,800 bp was present, suggesting an insertion. The lack of fragment 1 or 6 in types C to G suggests that these transposons had deletions from the left end. Furthermore, the lack of fragment 2 in types D and G suggests polymorphism at the right end of the transposon. No polymorphism was found among the restriction fragments from the central regions of Tn*1546*, *vanR*, *vanS*, *vanH*, and *vanA*. The high-molecular-mass bands present in types A to E and G represent DNA fragments flanking the VanA transposon. The absence of flanking fragments in type F is partially explained by deletions from the left end of the transposon (see above). In addition, the flanking fragment at the right end appeared to migrate at the position of fragment 4, while the original fragment 4 in lane F was absent, probably due to a rearrangement in this region.

Sequence analysis of the VanA transposons of representatives of the seven RFLP types. Thirteen representatives of the seven different Tn*1546* RFLP types (strains 3, 17, 22, 23, 24, 26, 42, 48, 51, 70, 74, 79, and 88 [Table 1]) were analyzed in more detail by determining the nucleotide sequence of the entire transposon. Overlapping internal fragments of Tn*1546* were amplified and were subsequently sequenced by using combinations of 35 Tn*1546*-specific primers (Table 2). The sequences that were obtained were compared with the published sequence of Tn*1546*. Consistent with the RFLP analysis, RFLP types C, D, E, F, and G lacked sequences at the left end of the transposon. In order to determine the exact left ends of the

TABLE 2. PCR and sequence primers used in this study

Primer[a]	Sequence	Positions[b]
Tn*1546* primers		
22.F	5′-GGATTTACAACGCTAAGCC	22–40
184.R	5′-ACCATATGTCGCCCTTAG	184–167
934.F	5′-TGTGGATTTGCATCTGC	934–950
1009.R	5′-ACGGTACAACATCTTCGTC	1009–991
1292.R	5′-TTACTCATGGATGTGGCC	1292–1275
1723.F	5′-ACAGGTGAGTCATCAGGC	1723–1740
1890.F	5′-TAAATAATCATAGTCGGCAGG	1890–1910
1913.R	5′-CGTCCTGCCGACTATG	1913–1898
1924.R	5′-TAGGAACTTGCACGTCCT	1924–1908
2768.F	5′-AGGATGGACTAACACCAATC	2768–2787
2880.R	5′-TGCTGTTCAATTAGCTGTTC	2880–2861
3514.F	5′-ACTGTAATGGCTGGTGTTAAC	3514–3534
3560.R	5′-TATCCGAATAAGATCTCGCT	3560–3542
3940.R	5′-ATTTATCAGATTATAGGGCCG	3940–3920
3992.F	5′-TTATTGTGGATGATGAACATG	3992–4012
4426.F	5′-AACGAGAAGCAGTTATCCC	4426–4444
4511.R	5′-TCGGAGCTAACCACATTC	4511–4494
5235.F	5′-ATATCACGTTGGACAAAGC	5235–5253
5374.R	5′-TTCATCGGTCATCTGCAC	5374–5357
5747.F	5′-ACGTTTAGGGTAGAGCTTCC	5747–5766
6039.F	5′-GTTTATGGATGTGAGCAGG	6039–6057
6113.R	5′-TATCGTTGCCATAACGC	6113–6097
6964.F	5′-AAAGGAGACAGGAGCATG	6964–6981
7035.R	5′-TTACGTCATGCTCCTCTGAG	7035–7017
7486.R	5′-CAAAAACAGGATAGGTAAACG	7486–7466
7875.F	5′-CCGCATTGTACTGAACG	7875–7891
7986.R	5′-CAAGCGGTCAATCAGTTC	7986–7969
8544.F	5′-GCATATAGCCTCGAATGG	8544–8561
8691.R	5′-TTACATACGTCGGGTTTCC	8691–8673
8969.R	5′-GATTGTGCCGTTTTGC	8969–8954
9519.F	5′-ACCAGCAGGTTATAGTGAGC	9519–9538
9580.R	5′-TCGTCAAGCTTGATCCTAC	9580–9562
9970.R	5′-GCCATCCTTACCTCCTTG	9970–9953
10716.R	5′-TTTTCCCCTCACTTCACAC	10716–10698
10778.F	5′-TTTAGTGCTGAGGAATTGG	10778–19796
IS*1216V* primers		
IS1216V.A	5′-GGAAAGCAATTTCAGCAG	254–271
IS1216V.B	5′-TCGATGCAGATGGTTTAAC	516–534
IS1216V.C	5′-CACTTGTAATAGAGGGGGC	659–641
IS1216V.D	5′-TGGGATTCCCAATAATACC	895–913
IS1216V.E	5′-AGCTTAAATCATAGATACCGTAAGG	913–935
IS1216V.F	5′-TTCATCGTCATTCCTCCTCCTG	243–225

[a] The names of the Tn*1546* primers indicate the position of the first nucleotide and the orientation of the primer (F, forward; R, reverse).

[b] The positions of the Tn*1546* primers are based on the sequence of Tn*1546* (GenBank and EMBL accession no. M97297). The positions of the IS*1216V* primers are based on the sequence of IS*1216V* (GenBank and EMBL accession no. L40841).

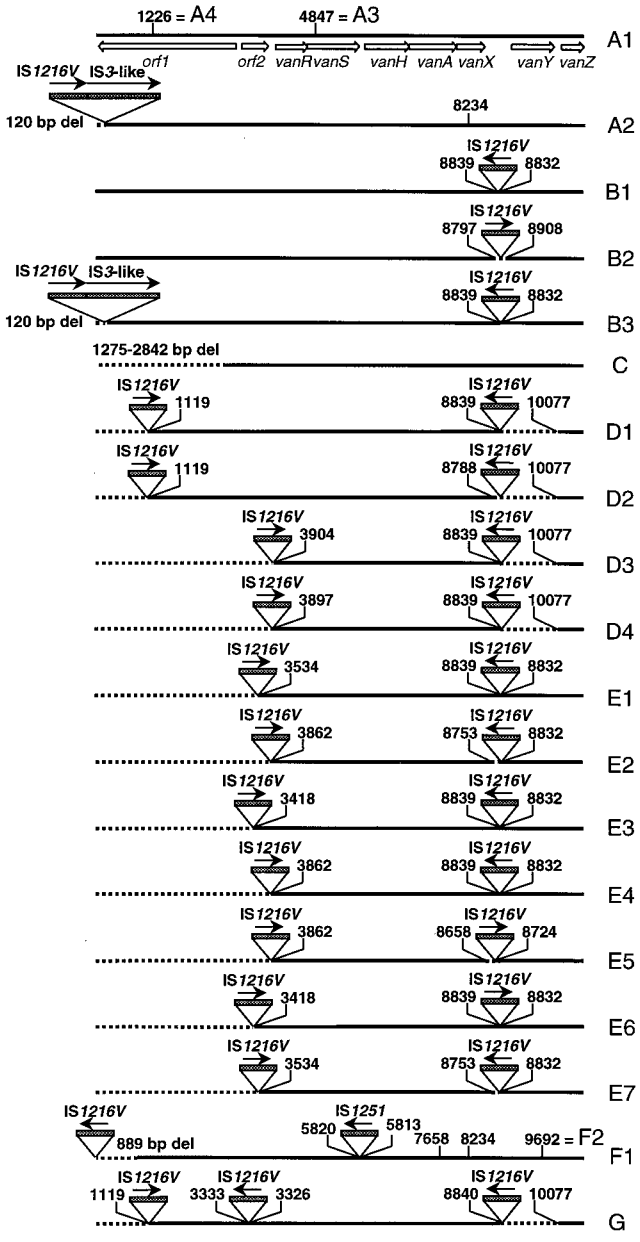

FIG. 2. Genetic maps of 22 Tn*1546* types. The thick horizontal lines represent the Tn*1546* types A1 to A4, B1 to B3, C, D1 to D4, E1 to E7, F1, F2, and G. The positions of genes and open reading frames (orf's) and the direction of transcription are depicted with open arrows. Dotted boxes represent IS elements. The positions of the first nucleotide upstream and the first nucleotide downstream from the IS insertion sites are depicted. Filled arrows indicate the transcriptional orientations of the inserted IS elements. Deletions (del) are indicated by dotted lines. The positions of base pair mutations are indicated above the different Tn*1546* types: 1226, T→A (K→stop); 4847, T→C; 7658, T→C (V→A); 8234, G→T (K→N); 9692, C→T (P→L).

truncated Tn*1546*-related elements, DNA fragments were amplified with a combination of Tn*1546*-derived primers and primers based on the insertion element IS*1216V*. IS*1216V* was found to be located upstream from Tn*1546* in strains of RFLP types D, E, F, and G. In strains of RFLP type C, no IS*1216V* was present upstream of the transposon, so that the exact left end of the transposon could not be determined and was estimated from the RFLP data to be between 1,275 and 2,842 bp.

The major rearrangements among the 13 strains investigated were the insertion of a IS*1216V*-IS*3*-like element at the left end of the transposon (types A2 and B3), the insertion of one or two copies of IS*1216V* (types B and D to G), the insertion of one copy of IS*1251* (type F), deletions associated with IS insertions downstream of *vanX* (types D to G), and at the left end of the transposon, deletions that affect the transposase or the resolvase gene (types C to G) (Fig. 2). Insertion of the IS*1216V*-IS*3*-like element at the left end of the transposon and insertion of IS*1216V* in the *vanXY* intergenic region have been described previously (3, 26). It is interesting that copies of IS*1216V* inserted in the *vanXY* intergenic region in strains 24, 42, 48, 70, and 74, which were completely sequenced, contained a synonymous T-to-C point mutation at position 826 relative to the published sequence of IS*1216V* (GenBank accession no. L40841). In all strains with IS*1216V* insertions except strains in which the IS insertions were accompanied by small adjacent deletions, an 8-bp duplication of the target sequence (CCCATTGT) was found. Insertion of IS*1216V* in the *vanXY* intergenic region also explained the presence of the additional 1.8-kbp fragment in types B, D, E, and G (Fig. 1). Insertion of IS*1251* in the *vanSH* intergenic region resulted in an 8-bp duplication of the target sequence, ATAATTTT. Furthermore, insertion of IS*1251* in this region explained the absence of fragment 4 in lane F (Fig. 1). Insertion of IS*1251* at

TABLE 3. Ribotypes, PFGE types, and Tn*1546* types of VRE isolated from the John Radcliffe Hospital, Oxford, and the Cook County Hospital, Chicago

Strain no.	Source	City	Ribotype[a]	PFGE type[b]	Tn*1546* type
48	Human stool	Oxford	2	G′	D1
49	Human stool	Oxford	4	U	D1
50	Human stool	Oxford	11	Q	D1
53	Human stool	Oxford	1	H′	D1
54	Human stool	Oxford	1	H	D1
55	Human urine	Oxford	1	I	D1
56	Human urine	Oxford	4	G	D2
57	Human urine	Oxford	5	P	D1
58	Human wound	Oxford	2	F	D1
59	Human blood	Oxford	6	P′	D1
60	Human urine	Oxford	6	H	D1
61	Human urine	Oxford	1	H	D1
62	Human blood	Oxford	1	H	D1
63	Human urine	Oxford	1	H	D1
64	Human urine	Oxford	1	H	D1
65	Human urine	Oxford	1	H	D1
66	Human urine	Oxford	1	H	D1
67	Human pus	Oxford	1	H″	D4
68	Human urine	Oxford	1	H	D4
69	Human pus	Oxford	1	H	D1
51	Human stool	Oxford	8	A	C
52	Human stool	Oxford	9	R	A1
88	Human[c]	Chicago	ND[d]	UU	F2
89	Human[c]	Chicago	ND	VV	F2
90	Human[c]	Chicago	ND	WW	F2
91	Human[c]	Chicago	ND	XX	F1
92	Human[c]	Chicago	ND	YY	F2
93	Human[c]	Chicago	ND	ZZ	F2
94	Human[c]	Chicago	ND	AAA	F2
95	Human[c]	Chicago	ND	BBB	F2
96	Human[c]	Chicago	ND	CCC	F2
97	Human[c]	Chicago	ND	DDD	F2

[a] Ribotypes have been described previously (31).
[b] Interpretation of banding patterns is according to Tenover et al. (45).
[c] The strains were from multiple body sites.
[d] ND, not determined.

this site has also been described previously (27). Furthermore, DNA polymorphism due to point mutations in *orf1* (1226), *vanS* (4847), *vanA* (7658), *vanX* (8234), and *vanY* (9692) were found (Fig. 2). Altogether 11 different Tn*1546* types were distinguished among the 13 strains whose transposons were sequenced: type A1 (which is Tn*1546*), A2, A3, A4, B1, C, D1, E1, E2, F2, and G (Fig. 2).

Analysis of the polymorphic regions in Tn*1546* in other isolates of VRE. We analyzed the polymorphic regions of Tn*1546* of 87 additional VRE which were initially examined by RFLP analysis. The presence of the point mutations in the *vanX*, *vanS*, and *orf1* genes, the exact integration sites and the orientations of IS*1216V* and IS*1251*, the deletions surrounding the IS*1216V* insertion site, and the size of the left-end deletion were assessed by means of DNA sequencing. Furthermore, isolates of VRE carrying the type F transposon were analyzed for the point mutation in the *vanA* and the *vanY* genes.

DNA sequencing finally distinguished 22 different transposon types. RFLP type A could be subdivided into four subtypes (subtypes A1 to A4), type B could be subdivided into three subtypes (subtypes B1 to B3), type D could be subdivided into four subtypes (subtypes D1 to D4), type E could be subdivided into seven subtypes (subtypes E1 to E7), and type F could be subdivided into two subtypes (subtypes F1 and F2). Types C and G could not be subdivided. On the basis of RFLP analysis, types D3 and D4 were initially designated E subtypes since they both lacked fragments 6, 1, and 3 at the left ends of their transposons. However, since these two types also lacked the *vanY* gene, which is indicative of type D, they were renamed D3 and D4. The identification of IS*1216V* in the *vanXY* intergenic region in types D1, D2, and D4 in strains 46 to 69 contradicts the results published previously by Jensen et al. (29) since in that study the same strains were partly analyzed, but no sequence or size variation was observed in the amplicons of the *vanXY* intergenic region.

Glycopeptide susceptibility patterns of isolates. The MICs of vancomycin, teicoplanin, and avoparcin for the 97 different isolates were determined by the agar dilution method. Generally, no association was found between the resistant phenotype and the transposon genotype. All isolates were resistant to vancomycin (MICs at which 50% [MIC_{50}] and 90% [MIC_{90}] of isolates are inhibited, 512 and 1,024 μg/ml, respectively) and avoparcin (MIC_{50} and MIC_{90}, 256 and 1,024 μg/ml, respectively). Exceptions were strains with deletions of the *vanY* gene (types D1, D2, D3, D4, and G). These strains were less resistant to teicoplanin (MIC_{50} and MIC_{90}, 16 and 64 μg/ml, respectively) than strains belonging to the other types (MIC_{50} and MIC_{90}, 128 and 256 μg/ml, respectively). It is conceivable that the deletion of *vanY* affects the transcription of *vanZ*, resulting in a lower MIC of teicoplanin, because *vanZ* has been shown to be involved in teicoplanin resistance (4, 48).

Tn*1546* types among VRE isolated from hospitalized patients. Our collection of VRE comprised two sets of strains isolated from hospitalized patients. One set of 22 VRE originated from an outbreak at the John Radcliffe Hospital in Oxford, United Kingdom (31). These 22 isolates represented eight different ribotypes and 13 different PFGE types (Table 3), which suggests that at least 13 different enterococcal strains were involved in this outbreak. In contrast, 17 of the 22 isolates contained the same D1 type of Tn*1546* (Table 3). Furthermore, an additional three strains harbored either Tn*1546* type D2 or Tn*1546* type D4, which could be derived from D1 by a single DNA rearrangement (Fig. 3). Tn*1546* type D1 was found among nine different strain types.

A second set of 10 strains originated from a 7-week survey for VRE contamination at Cook County Hospital, Chicago, Ill. (11, 12). All 10 *E. faecium* strains had different PFGE types (Table 3). Interestingly, all isolates except one contained the same Tn*1546* derivative, Tn*1546* type F2. One isolate, isolate VS4, contained the type F1 transposon, which differed from type F2 by a single base pair.

The data on the prevalence of transposon types in the Oxford and Chicago hospitals suggest the possibility of horizontal transmission of vancomycin resistance transposon types D1 and F, respectively, among different enterococcal hosts.

TABLE 4. Distribution of 22 different Tn*1546* derivatives among 97 isolates of VRE from human and animal sources

Tn*1546* type	No. of isolates from the following sources[a]:					
	Human (NL) (n = 21)	Animal (NL) (n = 24)	Human (UK) (n = 24)	Animal (UK) (n = 18)	Human (USA) (n = 10)	Total
A1	7	5	2	6		20
A2	13	12		4		29
A3		1				1
A4		1				1
B1		1		1		2
B2		1				1
B3				1		1
C			1			1
D1			17			17
D2			1			1
D3				1		1
D4			2			2
E1				1		1
E2		1	1			2
E3		1		1		2
E4				1		1
E5		1				1
E6	1					1
E7				1		1
F1					1	1
F2					9	9
G				1		1
Total	21	24	24	18	10	97

[a] NL, The Netherlands; UK, United Kingdom; USA, United States; n, total number of isolates from that source.

DISCUSSION

To facilitate understanding of the molecular epidemiology of vancomycin resistance, we undertook a detailed study of the molecular diversity and the evolutionary relationships of Tn*1546*-like elements in enterococci from humans and animals. Knowledge of the diversity of Tn*1546* is important for distinguishing between the dissemination of a single VRE clone and the transmission of a particular Tn*1546* type through a genetically divergent population of enterococci. Typing of VRE by methods such as PFGE and ribotyping has shown the clonal dissemination of VRE in hospitals (9, 25, 35, 40). However, transmission of particular Tn*1546* types has not been documented before. Nevertheless, various studies suggest that this occurs since genetic divergence in VRE genomes was found among strains isolated from epidemics caused by VRE (10, 15, 24, 35, 38).

In this study we have identified and characterized polymorphic regions in Tn*1546*-like elements from 97 VRE originating from animal and human sources. By means of a combination of RFLP analysis and DNA sequencing, 22 different Tn*1546*-like elements were distinguished. Three types of polymorphisms were found: point mutations, insertions of IS elements, and

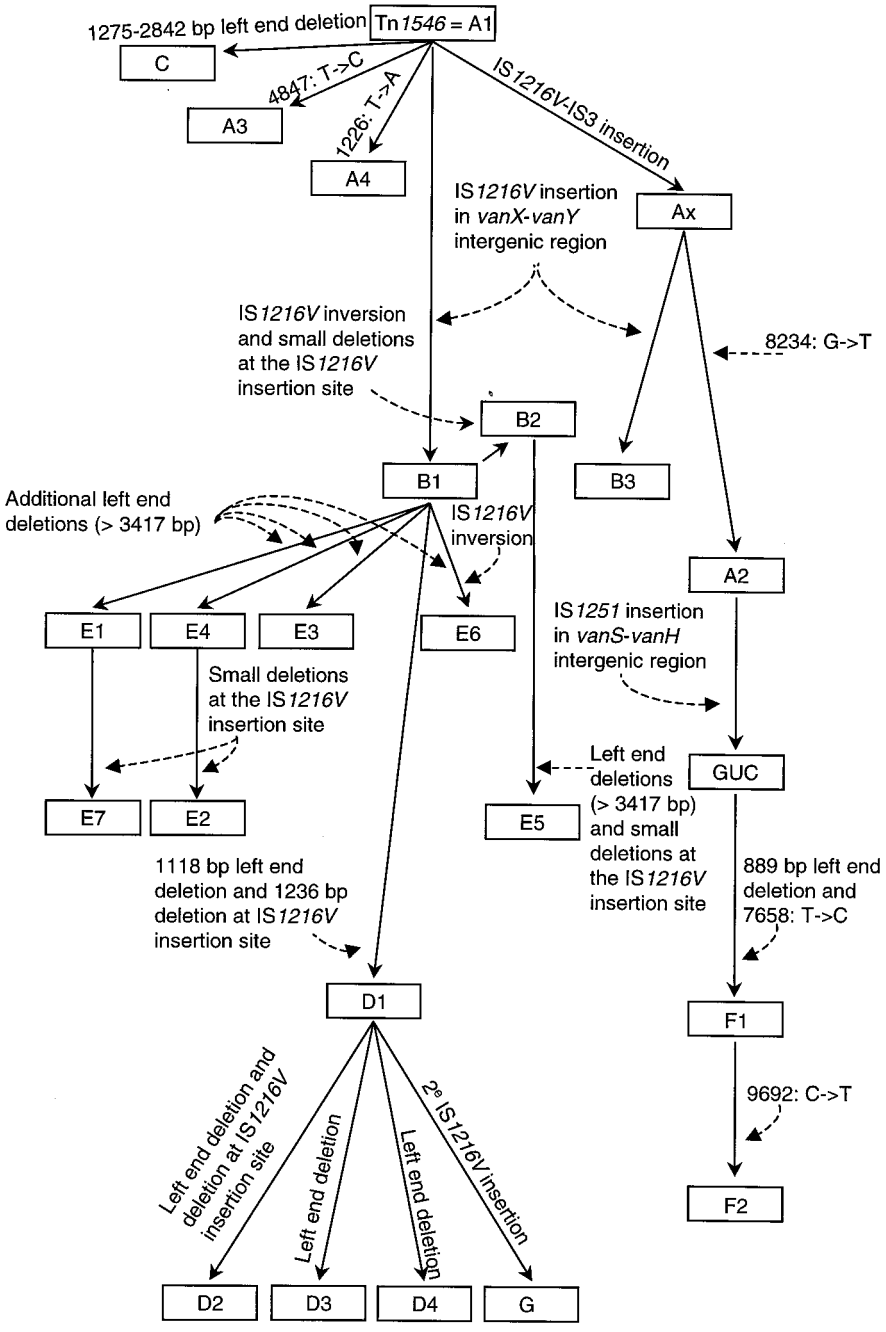

FIG. 3. Hypothetical evolutionary scheme for the various Tn*1546* derivatives characterized in this study from the archetypal transposon Tn*1546* (type A1) as described by Arthur et al. in 1993 (5). Boxes represent the different Tn*1546* types. Filled arrows indicate the transition of Tn*1546* type A1 to the other Tn*1546* types. The different DNA rearrangements, insertions, deletions, and point mutations are indicated. Strain GUC has been described by Handwerger et al. (27). 2°, secondary.

deletions generally associated with the insertion of IS elements. The point mutations were located in the *orf1*, *vanS*, *vanA*, *vanX*, and *vanY* genes. The only point mutation described previously is in the *vanX* gene at position 8234 (29, 48). Jensen et al. (29) also found this mutation in the *vanX* gene in three strains which we have also analyzed, strains 77 to 79.

The vast majority (74 of 97) of strains contained one to three copies of the insertion sequence IS*1216V* inserted in the vancomycin resistance transposon. Insertion of this IS element in the *vanXY* intergenic region and its presence on either side of Tn*1546* have been described previously (3, 26, 28). The presence of IS element insertions was often associated with deletions, a phenomenon which has been described previously (30, 52). Thirty isolates containing the type A2 and B3 VanA transposons had similar genetic organizations at the left end of the VanA transposon, as in strain GUC described by Handwerger and Skoble (26). In these types as well as in strain GUC, a copy of an IS*1216V*-IS*3* like element is present at the left end of the VanA transposon, resulting in a deletion of the first 120 bp. In strain GUC the Tn*1546*-like element is located on a large

chromosomal mobile element designated Tn*5482*. Preliminary analysis of two representative isolates carrying type A2 transposons indicated a chromosomal location of the VanA element (data not shown), which is similar to the case for strain GUC, which may suggest that type A2 and B3 VanA transposons are part of a larger chromosomal mobile element. In strains 77 to 79 the presence of the IS*1216V*-IS*3* element at the left end of the Tn*1546*-like element is consistent with the finding of Jensen et al. (29). In addition to IS*1216V*, insertions of IS*1251* in the *vanSH* intergenic region were found. Although the insertion of IS*1251* at this site was published previously, the transposon in *E. faecium* GUC described by Handwerger and colleagues (26, 27) was clearly distinct from the Tn*1546* type F transposon, since no insertion of an IS*1216V*-IS*3* like element was present directly upstream from Tn*1546* in the type F transposons.

Remarkable was the finding that 72 (74%) of the analyzed strains (types A2, B3, C, D1 to D4, E1 to E7, F1, F2, and G) carried small or large deletions in the transposase and resolvase regions of the Tn*1546*-like transposon. A similar finding has recently been reported by others (55). Although it is expected that deletions in the transposase and resolvase regions which abolish transposition may affect the dissemination of truncated Tn*1546*-like elements, other studies have shown that Tn*1546*-like elements are often part of chromosomal mobile elements (26) or plasmids that can be mobilized (28).

In this study we investigated in detail the polymorphism in Tn*1546* with the aim of exploiting differences in this genetic element for future studies on the epidemiology of vancomycin resistance. Because we examined a large number of strains from a variety of sources, some preliminary conclusions may be drawn. Tn*1546* types A1 and A2 were the most prevalent in The Netherlands both among isolates from humans and among isolates from farm animals (Table 4), suggesting an epidemiological link between animal and human reservoirs. The presence of identical VanA transposons in VRE isolated from humans and animals has also been described recently in Denmark and the United Kingdom (29, 55). In VRE from hospitalized patients in the United States we found transposons which contain insertions of IS*1251*. So far this IS element was been found only by Handwerger et al. (27), Jensen et al. (29), and MacKinnon et al. (34) in isolates from U.S. patients.

It is intriguing that the majority of the transposon types found in hospitals in the United Kingdom and the United States (types D1 and F2) have no counterpart in animals. For the U.S. isolates, this is explained by the fact that so far no VRE have been isolated from animals in the United States. The fact that no D types were found among the isolates from animals in the United Kingdom may suggest that once it was introduced in the Oxford hospital the VanA transposon has evolved independently from the transposons from counterpart strains from animals. This is consistent with the scheme presented in Fig. 3. Figure 3 depicts a hypothetical evolutionary scheme in an attempt to explain the relationships between the 22 transposon types. In Fig. 3 transposon types D (types D1, D2, and D4) and F (types F1 and F2) are located separately from the majority of the subtypes found outside hospitals. In the scheme presented in Fig. 3 we assume that the various Tn*1546* variants evolved by base pair substitutions, transpositions, and deletions. We did not include homologous recombination events, although they could lead to a more parsimonious phylogeny. The preliminary data on region specificity suggest that geographic isolation contributed to differences in the prevalence of particular Tn*1546* subtypes at different geographic sites.

The combination of the polymorphism in Tn*1546* and the epidemiological data indicate that the DNA polymorphism among Tn*1546* variants can be exploited successfully for the tracing of the routes of transmission of vancomycin resistance genes. Indicative of this is the finding of identical or closely related VanA transposon types among genetically different enterococci in the Oxford hospital as well as in the hospital in Chicago. Studies are in progress to use the tools developed in this study to investigate in detail the prevalence of subtypes of Tn*1546* among humans and animals. This may resolve the controversial issue of the spillover of vancomycin resistance to humans from the animal reservoir due to the use in animal husbandry of glycopeptide antibiotics, such as avoparcin, for growth promotion. Avoparcin has been used in Europe for more than 20 years, but it is anticipated that the current ban on the veterinary use of this antibiotic will also lead to an overall decrease in the frequency of vancomycin resistance among enterococci colonizing the human digestive tract.

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REFERENCES

1. **Aarestrup, F. M.** 1995. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolated from conventional and ecological poultry farms. Microb. Drug Resist. **1:**255–257.
2. **Aarestrup, F. M., P. Ahrens, M. Madsen, L. V. Pallesen, R. L. Poulsen, and H. Westh.** 1996. Glycopeptide susceptibility among Danish *Enterococcus faecium* and *Enterococcus faecalis* isolates of animal and human origin and PCR identification of genes within the VanA cluster. Antimicrob. Agents Chemother. **40:**1938–1940.
3. **Arthur, M., F. Depardieu, G. Gerbaud, M. Galimand, R. Leclercq, and P. Courvalin.** 1997. The VanS sensor negatively controls VanR-mediated transcriptional activation of glycopeptide resistance genes of Tn*1546* and related elements in the absence of induction. J. Bacteriol. **179:**97–106.
4. **Arthur, M., F. Depardieu, C. Molinas, P. Reynolds, and P. Courvalin.** 1995. The *vanZ* gene of Tn*1546* from *Enterococcus faecium* BM4147 confers resistance to teicoplanin. Gene **154:**87–92.
5. **Arthur, M., C. Molinas, F. Depardieu, and P. Courvalin.** 1993. Characterization of Tn*1546*, a Tn*3*-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J. Bacteriol. **175:**117–127.
6. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and L. Struhl.** 1989. Current protocols in molecular biology, section 2.4. Greene Publishing Associates, Brooklyn, N.Y.
7. **Bager, F., M. Madsen, J. Christensen, and F. M. Aarestrup.** 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. Prev. Vet. Med. **31:**95–112.
8. **Bates, J., J. Z. Jordens, and D. T. Griffiths.** 1994. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J. Antimicrob. Chemother. **34:**507–514.
9. **Biavasco, F., A. Miele, C. Vignaroli, E. Manso, R. Lupidi, and P. E. Varaldo.** 1996. Genotypic characterization of a nosocomial outbreak of VanA *Enterococcus faecalis*. Microb. Drug Resist. **2:**231–237.
10. **Bingen, E. H., E. Denamur, N. Y. Lambert-Zechovsky, and J. Elion.** 1991. Evidence for the genetic unrelatedness of nosocomial vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. J. Clin. Microbiol. **29:** 1888–1892.
11. **Bonten, M. J. M., M. K. Hayden, C. Nathan, T. W. Rice, and R. A. Weinstein.** 1998. Stability of vancomycin-resistant enterococcal genotypes isolated from long-term-colonized patients. J. Infect. Dis. **177:**378–382.
12. **Bonten, M. J. M., M. K. Hayden, C. Nathan, J. van Voorhis, M. Matushek,**

S. Slaughter, T. Rice, and R. A. Weinstein. 1996. Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. Lancet **348:**1615–1619.
13. **Centers for Disease Control and Prevention.** 1993. Nosocomial enterococci resistant to vancomycin—United States, 1989–1993. Morbid. Mortal. Weekly Rep. **42:**597–599.
14. **Chadwick, P. R., N. Woodford, E. B. Kaczmarski, S. Gray, R. A. Barrell, and B. A. Oppenheim.** 1996. Glycopeptide-resistant enterococci isolated from uncooked meat. J. Antimicrob. Chemother. **38:**908–909.
15. **Clark, N. C., R. C. Cooksey, B. C. Hill, J. M. Swenson, and F. C. Tenover.** 1993. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob. Agents Chemother. **37:**2311–2317.
16. **Coque, T. M., J. F. Tomayko, S. C. Ricke, P. C. Okhyusen, and B. E. Murray.** 1996. Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. Antimicrob. Agents Chemother. **40:** 2605–2609.
17. **Devriese, L. A., M. Ieven, H. Goossens, P. Vandamme, B. Pot, J. Hommez, and F. Haesebrouck.** 1996. Presence of vancomycin-resistant enterococci in farm and pet animals. Antimicrob. Agents Chemother. **40:**2285–2287.
18. **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.)
19. **Eliopoulos, G. M., C. B. Wennersten, H. S. Gold, T. Schulin, M. Souli, M. G. Farris, S. Cerwinka, H. L. Nadler, M. Dowzicky, G. H. Talbot, and R. C. Moellering.** 1998. Characterization of vancomycin-resistant *Enterococcus faecium* isolates from the United States and their susceptibility in vitro to dalfopristin-quinupristin. Antimicrob. Agents Chemother. **42:**1088–1092.
20. **Endtz, H., N. van den Braak, A. van Belkum, J. A. J. W. Kluytmans, J. G. M. Koeleman, L. Spanjaard, A. Voss, A. J. L. Weersink, C. M. J. E. Vandenbroucke-Grauls, A. G. M. Buiting, A. van Duin, and H. A. Verbrugh.** 1997. Fecal carriage of vancomycin-resistant enterococci in hospitalized patients and those living in the community in The Netherlands. J. Clin. Microbiol. **35:** 3026–3031.
21. **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.
22. **Gordts, B., H. Van Landuyt, M. Ieven, P. Vandamme, and H. Goossens.** 1995. Vancomycin-resistant enterococci colonizing the intestinal tracts of hospitalized patients. J. Clin. Microbiol. **33:**2842–2846.
23. **Haaheim, H., K. H. Dahl, G. S. Simonsen, O. Olsvik, and A. Sundsfjord.** 1998. Long PCRs of transposons in the structural analysis of genes encoding acquired glycopeptide resistance in enterococci. BioTechniques **24:**432–437.
24. **Hall, L. M., B. Duke, M. Guiney, and R. Williams.** 1992. Typing of *Enterococcus* species by DNA restriction fragment analysis. J. Clin. Microbiol. **30:** 915–919.
25. **Handwerger, S., B. Raucher, D. Altarac, J. Monka, S. Marchione, K. V. Singh, B. E. Murray, J. Wolff, and B. Walters.** 1993. Nosocomial outbreak due to *Enterococcus faecium* highly resistant to vancomycin, penicillin, and gentamicin. Clin. Infect. Dis. **16:**750–755.
26. **Handwerger, S., and J. Skoble.** 1995. Identification of chromosomal mobile element conferring high-level vancomycin resistance in *Enterococcus faecium*. Antimicrob. Agents Chemother. **39:**2446–2453.
27. **Handwerger, S., J. Skoble, L. F. Discotto, and M. J. Pucci.** 1995. Heterogeneity of the *vanA* gene cluster in clinical isolates of enterococci from the northeastern United States. Antimicrob. Agents Chemother. **39:**362–368.
28. **Heaton, M. P., L. F. Discotto, M. J. Pucci, and S. Handwerger.** 1996. Mobilization of vancomycin resistance by transposon-mediated fusion of a VanA plasmid with an *Enterococcus faecium* sex pheromone-response plasmid. Gene **171:**9–17.
29. **Jensen, L. B., P. Ahrens, L. Dons, R. N. Jones, A. M. Hammerum, and F. M. Aarestrup.** 1998. Molecular analysis of Tn*1546* in *Enterococcus faecium* isolated from animals and humans. J. Clin. Microbiol. **36:**437–442.
30. **Jilk, R. A., J. C. Makris, L. Borchardt, and W. S. Reznikoff.** 1993. Implications of Tn*5*-associated adjacent deletions. J. Bacteriol. **175:**1264–1271.
31. **Jordens, J. Z., J. Bates, and D. T. Griffiths.** 1994. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J. Antimicrob. Chemother. **34:**515–528.
32. **Klare, I., H. Heier, H. Claus, G. Bohme, S. Marin, G. Seltmann, R. Hakenbeck, V. Antanassova, and W. Witte.** 1995. *Enterococcus faecium* strains with *vanA*-mediated high level glycopeptide resistance isolated from animal foodstuffs and faecal samples of humans in the community. Microb. Drug Resist. **1:**265–272.
33. **Klare, I., H. Heier, H. Claus, R. Reissbrodt, and W. Witte.** 1995. *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol. Lett. **125:**165–171.
34. **MacKinnon, M. G., M. A. Drebot, and G. J. Tyrrell.** 1997. Identification and characterization of IS*1476*, an insertion sequence-like element that disrupts VanY function in a vancomycin-resistant *Enterococcus faecium* strain. Antimicrob. Agents Chemother. **41:**1805–1807.
35. **Mato, R., H. Delencastre, R. B. Roberts, and A. Tomasz.** 1996. Multiplicity of genetic backgrounds among vancomycin-resistant *Enterococcus faecium* isolates recovered from an outbreak in a New York City Hospital. Microb. Drug Resist. **2:**309–317.
36. **McDonald, L. C., M. J. Kuehnert, F. C. Tenover, and W. R. Jarvis.** 1997. Vancomycin-resistant enterococci outside the health-care setting: prevalence, sources, and public health implications. Emerg. Infect. Dis. **3:**311–317.
37. **Miele, A., M. Bandera, and B. P. Goldstein.** 1995. Use of primers selective for vancomycin resistance genes to determine *van* genotype in enterococci and to study gene organization in VanA isolates. Antimicrob. Agents Chemother. **39:**1772–1778.
38. **Morris, J. G., Jr., D. K. Shay, J. N. Hebden, R. J. McCarter, Jr., B. E. Perdue, W. Jarvis, J. A. Johnson, T. C. Dowling, L. B. Polish, and R. S. Schwalbe.** 1995. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establishment of endemicity in a university medical center. Ann. Intern. Med. **123:**250–259.
39. **Noble, W. C., Z. Virani, and R. G. Cree.** 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS Microbiol. Lett. **72:**195–198.
40. **Pegues, D. A., C. F. Pegues, P. L. Hibberd, D. S. Ford, and D. C. Hooper.** 1997. Emergence and dissemination of a highly vancomycin-resistant *vanA* strain of *Enterococcus faecium* at a large teaching hospital. J. Clin. Microbiol. **35:**1565–1570.
41. **Power, E. G., Y. H. Abdulla, H. G. Talsania, W. Spice, S. Aathithan, and G. L. French.** 1995. *vanA* genes in vancomycin-resistant clinical isolates of *Oerskovia turbata* and *Arcanobacterium* (*Corynebacterium*) *haemolyticum*. J. Antimicrob. Chemother. **36:**595–606.
42. **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.
43. **Schouten, M. A., J. A. A. Hoogkamp-Korstanje, and A. Voss.** 1997. Controlling glycopeptide-resistant enterococci. Clin. Microbiol. Infect. **3:**592–593.
44. **Suppola, J. P., L. Volin, V. V. Valtonen, and M. Vaara.** 1996. Overgrowth of *Enterococcus faecium* in the feces of patients with hematologic malignancies. Clin. Infect. Dis. **23:**694–697.
45. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
46. **Torres, C., J. A. Reguera, M. J. Sanmartin, J. C. Perez-Diaz, and F. Baquero.** 1994. *vanA*-mediated vancomycin-resistant *Enterococcus* spp. in sewage. J. Antimicrob. Chemother. **33:**553–561.
47. **Van Belkum, A., N. van den Braak, R. Thomassen, H. Verbrugh, and H. Endtz.** 1996. Vancomycin-resistant enterococci in cats and dogs. Lancet **348:** 1038–1039.
48. **Van den Bogaard, A. E., L. B. Jensen, and E. E. Stobberingh.** 1997. Vancomycin-resistant enterococci in turkeys and farmers. N. Engl. J. Med. **337:** 1558–1559.
49. **Van den Bogaard, A. E., P. Mertens, N. H. London, and E. E. Stobberingh.** 1997. High prevalence of colonization with vancomycin- and pristinamycin-resistant enterococci in healthy humans and pigs in The Netherlands: is the addition of antibiotics to animal feeds to blame? J. Antimicrob. Chemother. **40:**454–456.
50. **Van den Braak, N., A. van Belkum, M. van Keulen, J. Vliegenthart, H. A. Verbrugh, and H. P. Endtz.** 1998. Molecular characterization of vancomycin-resistant enterococci from hospitalized patients and poultry products in The Netherlands. J. Clin. Microbiol. **36:**1927–1932.
51. **Van der Auwera, P., N. Pensart, V. Korten, B. E. Murray, and R. Leclercq.** 1996. Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly glycopeptide-resistant enterococci. J. Infect. Dis. **173:** 1129–1136.
52. **Wang, G., X. Xu, J. M. Chen, D. E. Berg, and C. M. Berg.** 1994. Inversions and deletions generated by a mini-gamma delta (Tn*1000*) transposon. J. Bacteriol. **176:**1332–1338.
53. **Wegener, H. C., M. Madsen, N. Nielsen, and F. M. Aarestrup.** 1997. Isolation of vancomycin resistant *Enterococcus faecium* from food. Int. J. Food Microbiol. **35:**57–66.
54. **Werner, G., I. Klare, and W. Witte.** 1997. Arrangement of the *vanA* gene cluster in enterococci of different ecological origin. FEMS Microbiol. Lett. **155:**55–61.
55. **Woodford, N., A.-M. A. Adebiyl, M.-F. I. Palepou, and B. D. Cookson.** 1998. Diversity of VanA glycopeptide resistance elements in enterococci from humans and nonhuman sources. Antimicrob. Agents Chemother. **42:**502–508.