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Molecular Analysis of Tn*1546*-Like Elements in Vancomycin-Resistant Enterococci Isolated from Patients in Europe Shows Geographic Transposon Type Clustering

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Resistance mechanism relatedness was studied in 18 clinical, European *vanA* vancomycin-resistant enterococci. Molecular analysis revealed 10 Tn*1546*-like elements, suggesting two evolutionary lineages. Lineage I dominated the European mainland, and lineage II dominated the United Kingdom and Israel. Geographic clustering reflected different types of meat consumption between countries, since each lineage is associated with colonization of different animals.

Over the past decade, vancomycin-resistant enterococci (VRE) have emerged worldwide (7, 10, 15). Prevalences vary between the United States and Europe, and a different epidemiology for these continents has been postulated (7, 15). Six vancomycin resistance types in enterococci have been described: VanA, VanB, VanC, VanD, VanE, and VanG (6, 17). VanF glycopeptide resistance has been described but has not yet been seen in enterococci (19). VanA type resistance, characterized by high-level inducible vancomycin resistance (MICs of 64 to >1,024 mg/liter) and teicoplanin resistance (MICs of 16 to >512 mg/liter), is most frequently encountered. VanA resistance results from VanA transposon Tn*1546* acquisition. Detailed molecular analysis of Tn*1546*-like elements in enterococci isolated from human and animal sources has revealed the presence of different Tn*1546* subtypes. These differences include point mutations, insertions of insertion sequence (IS) elements, and deletions (2–4, 8, 9, 11, 12, 14, 18, 21–25, 27–29; A. L. da Costa Darini, M.-F. I. Palepou, D. James, and N. Woodford, Letter, Antimicrob. Agents Chemother. **43:**995–996, 1999; L. B. Jensen, Letter, Antimicrob. Agents Che-

TABLE 1. Strain origin, species, Tn*1546* type, and patient data

Strain no.	Tn*1546* type	Country	City	Species	Sex[a]	Age group[b]	Department[c]	Isolation site[d]
4	A1	Germany	Aachen	*E. faecium*	M	A	Urology	Urogenital tract
2	A1	Czech Republic	Prague	*E. faecium*	F	G	Internal medicine	Urogenital tract
11	A1	Italy	Torino	*E. faecium*	F	G	Other	Urogenital tract
12	A1	Italy	Torino	*E. faecium*	F	A	OPD	Digestive tract
13	A1	Italy	Catania	*E. faecium*	F	A	Hematology	Skin
14	A1	Slovak Republic	Bratislava	*E. faecium*	F	N	Intensive care	Digestive tract
5	A5	Germany	Frankfurt am Main	*E. faecium*	M	A	Hematology	Digestive tract
6	A5	Germany	Frankfurt am Main	*E. faecium*	M	A	OPD	Skin
3	A6	France	Paris	*E. faecium*	M	C	Pediatrics	Digestive tract
18	B3	United Kingdom	London	*E. faecium*	F	C	Other	Blood
1	B4	Belgium	Brussels	*E. faecalis*	M	A	OPD	Respiratory tract
16	C	United Kingdom	Manchester	*E. faecium*	M	A	General practitioner	Other
10	E2	Israel	Beer-Sheva	*E. faecium*	F	A	Internal medicine	Skin
9	E4	Israel	Jerusalem	*E. faecium*	F	G	Other	Urogenital tract
17	E4	United Kingdom	London	*E. faecium*	M	C	Intensive care	Other
15	E12	United Kingdom	Manchester	*E. faecium*	F	G	Other	Other
7	E13	Israel	Jerusalem	*E. faecalis*	M	A	Surgery	Skin
8	E13	Israel	Jerusalem	*E. faecium*	F	G	Surgery	Blood

[a] M, male; F, female.
[b] N, less than 1 year old; C, between 1 and 16 years old; A, between 16 and 65 years old; G, 65 years and older.
[c] OPD, outpatient department; Other, not further specified.
[d] Other, not further specified.

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mother. **42:**2463–2464, 1998; L. B. Jensen, A. M. Hammerum, R. L. Poulsen, and H. Westh, Letter, Antimicrob. Agents Chemother. **43:**724–725, 1999; G. S. Simonsen, K. H. Dahl, M. R. Mikalsen, O. Ølsvik, and A. Sundsfjord, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother, abstr. C-82, p. 92, 1998). Although genetic diversity of Tn*1546* like elements has been described in great detail by several authors, the different Tn*1546* subtypes, which were found in different European studies, are difficult to compare, since various molecular techniques were used (11, 12, 18, 21, 24, 27, 29; Jensen, Letter).

The aim of this study was to investigate the genetic relationships between the *vanA* transposons of VRE from different European countries.

Early in 1997, 4,208 clinical enterococcal strains were collected by 49 centers representing 27 European countries (20; M. A. Schouten, J. A. A. Hoogkamp-Korstanje, C. J. M. Bartels, H. J. G. R. Roelofs-Willemse, Y. J. M. Peters, and A. Voss, Abstr. Eighth Annu. Meet. Soc. Healthcare Epidemiol. Am., abstr. 18, p. 28, 1998). Fifty-one strains exhibiting vancomycin resistance were characterized for the presence of the *vanA*, *vanB*, and *vanC* genes by means of a multiplex PCR as described by Dutka-Malen et al. (5). In 18 isolates, the *vanA* gene was detected, while *vanB* and *vanC* genes were detected in 5 and 28 isolates, respectively. This means that the overall prevalence of VRE among clinical European isolates is still very low (Schouten et al., Abstr. Eighth Annu. Meet. Soc. Healthcare Epidemiol. Am., 1998).

Detailed molecular characterization of Tn*1546*-like elements in the 18 VanA VRE was performed by a combination of restriction fragment length polymorphism (RFLP) analysis and DNA sequencing of internal PCR fragments of VanA transposons as described previously (27). All VRE were analyzed for the presence of point mutations at positions 1226, 4847, 7658, 8234, and 9692; insertions of IS*1216V* in the *vanX-vanY* intergenic region; and left-end deletions (26, 27). The exact integration site and orientation of the IS*1542* insertion in the *orf2-vanR* intergenic region were determined by amplifying and sequencing a DNA fragment with the primer combination IS1216V.E (5′-AGCTTAAATCATAGATACCGTAAGG)-Tn*1546* 4511.R (5′-TCGGAGCTAACCACATTC). Strain origin, species, Tn*1546* type, and epidemiological data concerning patients from whom strains were isolated are shown in Table 1.

Among 18 VanA VRE, 10 different Tn*1546*-like elements could be distinguished (Fig. 1). A scheme was constructed that describes the hypothetical evolutionary relationship between Tn*1546* variants found in this study and those found in earlier studies (Fig. 2). This scheme is comparable to the scheme described previously (27). Six isolates from Italy, regardless of the center of origin, Czech Republic, and the Slovak Republic contained type A1 transposon (Table 1), which is identical to transposon Tn*1546*, as described by Arthur et al. (1). This confirms earlier findings that VanA transposons indistinguishable from Tn*1546* are frequently encountered in Europe (12, 18, 21, 27, 29; Jensen, Letter; Simonsen et al., 38th ICAAC). From type A1, two main lineages of Tn*1546* derivatives may have evolved. Lineage I includes the types A2 (not found in this study), A5, and A6 and is characterized by the point mutation at 8234 in *vanX*. Types A5, encountered twice in Germany, and A6, present in one isolate from France, are

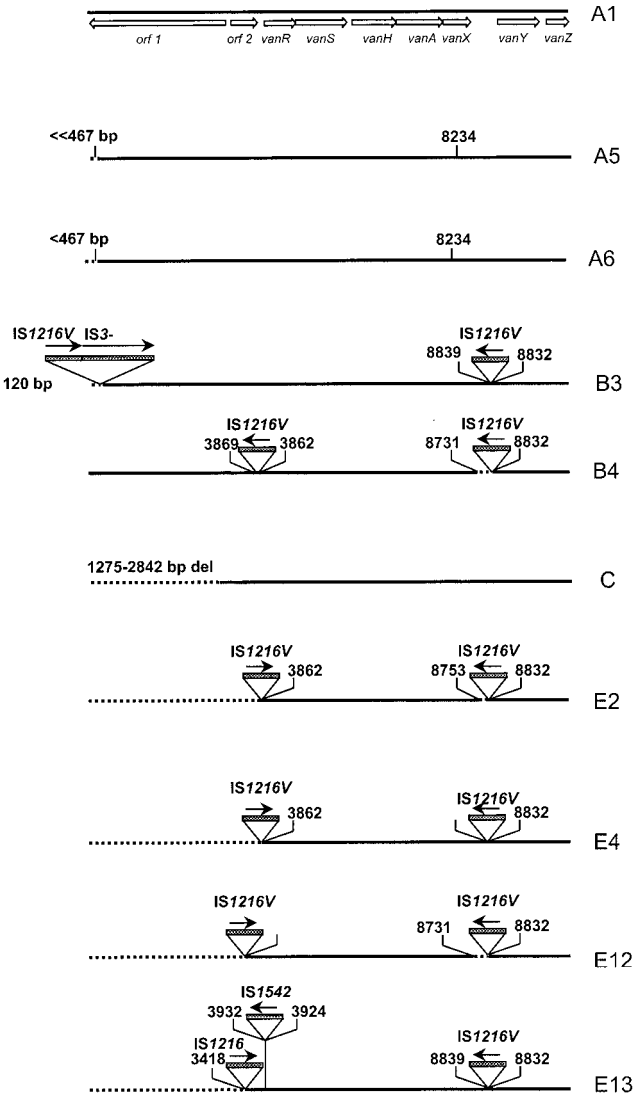

FIG. 1. Genetic maps of 10 Tn*1546* types. The thick horizontal lines represent the different Tn*1546* types. The positions of genes and open reading frames (*orf1* and *orf2*) and the direction of transcription are depicted with open arrows. Dotted boxes represent IS elements. The positions of the first nucleotide upstream and the first nucleotide downstream from the IS insertion sites are depicted. Solid arrows indicate the transcriptional orientation of the inserted IS elements. Deletions (del) are indicated by dotted lines. The position of the base pair mutation at 8234, G→T (K→N), is indicated above the A5 and A6 Tn*1546* types.

closely related. They both contain, in addition to the G→T point mutation in the *vanX* gene at position 8234, a small deletion at the left end of the transposon. Although the exact size of the deletion in the types A5 and A6 could not be determined, the RFLP and PCR results revealed that the deletion in type A5 has to be close to the *Hae*III restriction site at position 467, while in type A6, the deletion is probably somewhat smaller than the deletion in A5. Lineage II types include the B and E types and are characterized by IS*1216V* insertions in the *vanX-vanY* intergenic region, often accompanied by small deletions adjacent to the insertion site, and

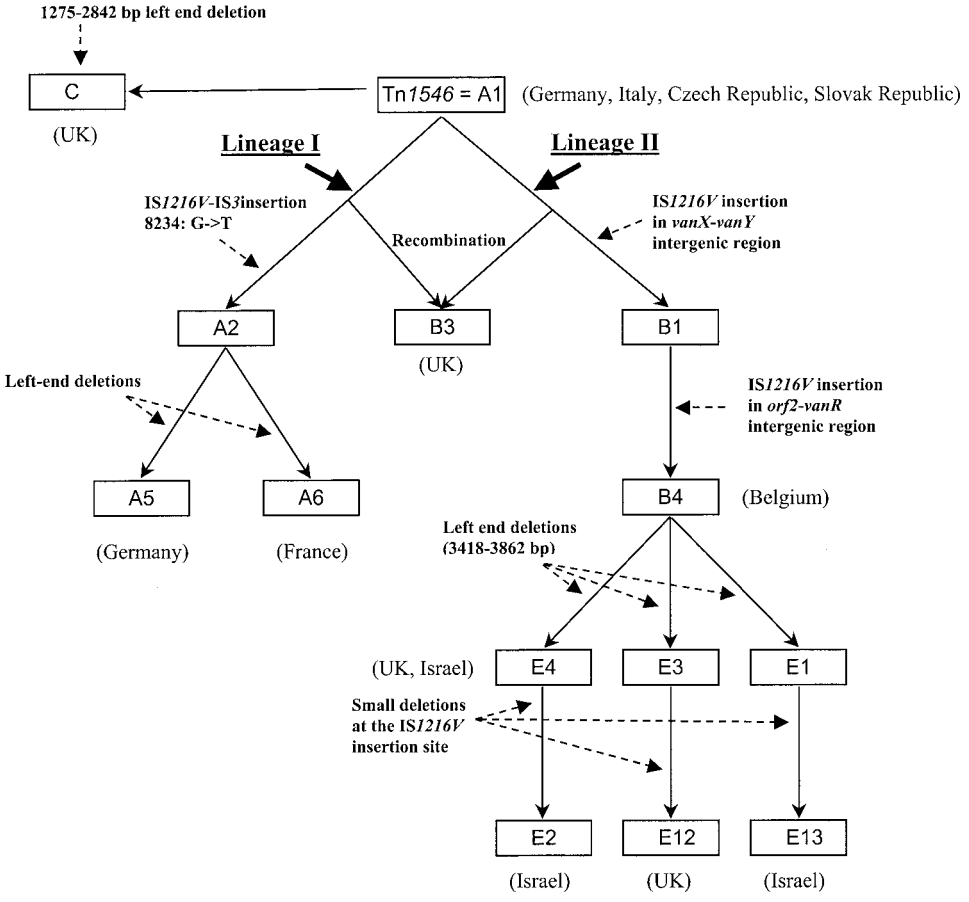

FIG. 2. Hypothetical evolutionary scheme for the various Tn*1546* derivatives characterized in this study from the archetypal transposon Tn*1546* (type A1) as described by Arthur et al. in 1993 (1). The types A2, B1, E1, and E3 were described previously by Willems et al. (27) and not in this study. They were included for better understanding of the different Tn*1546* type evolutionary relationships. Boxes represent the different Tn*1546* types. Solid arrows indicate the transition of Tn*1546* type A1 to the other Tn*1546* types. The two different lineages, I and II, are indicated. The names of the countries where the different transposon types were found are indicated in parentheses. This scheme was partly based on the evolutionary scheme described by Willems et al. (27).

insertions of IS*1216V* at the left end of the transposon associated with large deletions encompassing the *orf1* and *orf2* region (Fig. 1 and 2). Transposon types of lineage II were found in isolates from Belgium (B4), the United Kingdom (types E4 and E12), and Israel (E2, E4, and E13). Strains 7 (*Enterococcus faecalis*) and 8 (*Enterococcus faecium*) from Israel both possess type E13, suggesting horizontal transfer of this transposon type between two different enterococcal species. Interestingly, in these two strains, the insertion element IS*1542* was inserted in the VanA transposon at exactly the same position as in the group H VanA transposon described by Woodford and colleagues (29). Nevertheless, type E13 is probably not identical to the group H transposon, because the left-end deletion in type E13 appears to be much larger than in the group H transposon. So far, IS*1542* has been found frequently in clinical and poultry VRE isolates from the United Kingdom and Ireland and sporadically in glycopeptide-sensitive enterococci from the United Kingdom and Brazil. The finding of lineage II transposon types in the United Kingdom is in agreement with results published previously by Woodford et al., who found that the majority of their human United Kingdom strains possessed a transposon that would most likely fit into our groups B and E (29). Finally, in one isolate from the United Kingdom, transposon type C was found. This transposon type was described previously (27).

It is interesting that the lineage II transposon types B and E, which are predominantly found in the United Kingdom and Israel, are also the types frequently found in poultry isolates (22, 24, 27, 29), while, e.g., in The Netherlands, type A2, a lineage I type, is the most prevalent type encountered in human and pig isolates (27). In Israel and the United Kingdom, the per capita consumption of poultry meat (34.8 and 19.2 kg, respectively) is about twofold higher than in The Netherlands (15.6 kg). On the other hand, the Dutch consume twice as much pork per capita as people in the United Kingdom (45.6 versus 25.6 kg, respectively) (http://usda.mannlib.cornell.edu/data-sets/food/91004/). Although no exact figures for the consumption of pork in Israel were available, the level is expected to be very low as a result of the kosher lifestyle. These data suggest that the geographical distribution of different transposon types may be a result of the differences in meat consumption in the different European countries, thus indicating that

vanA transposons found in humans in Europe originate from farm animals.

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