

Letter to the Editor

Differences in the Occurrence of Two Base Pair Variants of *Tn1546* from Vancomycin-Resistant Enterococci from Humans, Pigs, and Poultry

In recent years the risk of transferring vancomycin-resistant enterococci (VRE) from animals to humans has caused great concern. Studies have shown that the glycopeptide growth promoter avoparcin selects for VRE (1, 3), and high numbers have been found in food animals and foodstuff in Europe (4, 7, 10). In one case, identical strains were isolated from both a turkey farmer and his turkeys (9). The finding of VRE in nonhospitalized humans and in meat eaters but not in vegetarians has further indicated a food-associated spread of vancomycin resistance from animals to humans (8). Most of the vancomycin-resistant *Enterococcus faecium* (VREF) strains isolated in Europe contain *Tn1546*. This transposon was first isolated from *E. faecium* BM4147 (2). Studies of *Tn1546* have revealed that only the *vanA*, *vanH*, and *vanX* genes are essential for resistance (Fig. 1). Characterization of *Tn1546*-like elements in isolates of animal and human origin indicated only minor variations caused by insertion sequences outside the essential part of *Tn1546* (Fig. 1), and a base pair variation in the *vanX* gene was discovered at position 8234 (5). In this position either a G (G type) or a T (T type) was found (Fig. 1). Based on all these variations, different types of *Tn1546*-like elements were defined, and indistinguishable elements were found in isolates of human and animal origin (5).

In this study a total of 271 VREF isolates of animal (226) and human (45) origin were investigated for this base pair variation. All animal isolates originated from different herds, and all human isolates, except strains from Norway and Saudi Arabia, have previously been typed to independent clones (4–6). PCR amplification of a 424-bp amplicon of the *vanX* gene of *Tn1546* (Fig. 1) was obtained from all isolates, confirming the presence of *Tn1546*-like elements. By digesting the amplicons with the restriction enzyme *DdeI*, two distinct patterns of fragments were obtained for the G and T types. Based on these results the distribution of the base pair variation could thus be ascertained.

As evident from Table 1, all isolates from poultry belonged to the G type, while 32 of the 33 porcine isolates belonged to the T type. In human isolates both variations were found but only one of the variants dominated locally. Only the G type was found in isolates from England, Norway, and Saudi Arabia, thus associating these isolates with poultry. For the human Norwegian isolates this could be expected, since they were isolated from poultry farmers. All Danish human VREF isolates belonged to the T type, thereby associating them with pigs.

The present study showed that VREF isolates from pigs, poultry, and humans could be divided according to base pair variation in the *vanX* gene at position 8234 (G or T type). All poultry isolates belonged to the G type, whereas almost all porcine isolates, except one Danish isolate, belonged to the T type. This finding indicates, with particular reference to the Danish isolates, that horizontal exchange of VREF isolates or *Tn1546*-like elements between poultry and pigs does not occur frequently. On the other hand, both types were found among humans, indicating that humans may be infected from both sources. Based on this observation it may be hypothesized that the primary transmission is from animals to humans and not the other way around. However, further studies are required to test this assertion. The present results indicate that the base pair variation in the *vanX* gene may be a useful marker for epidemiological studies on the spread of VREF isolates and vancomycin resistance genes.

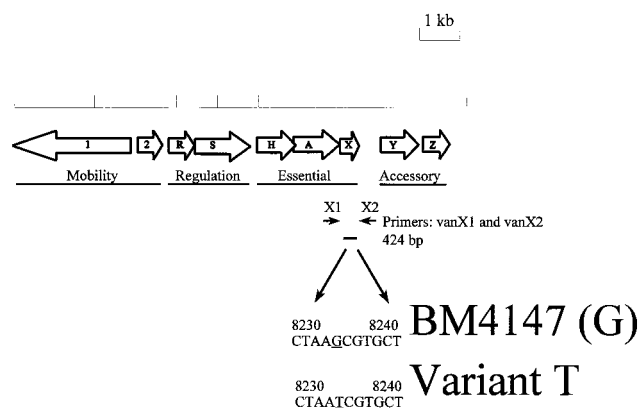


FIG. 1. The *Tn1546* encoding vancomycin resistance. The sizes and positions of the genes are indicated. The genes are grouped into the categories Mobility, Regulation, Essential, and Accessory according to their importance for vancomycin resistance. The positions and names of the primers used for amplification of an internal area of *vanX* are indicated as is the size for the obtained amplicon. Sequences from position 8230 to 8240 for the G and T types are shown with the base pair variation underlined. The sequence for the G-type variation is from the first published sequence for strain BM4147.

TABLE 1. Variations in *Tn1546*-like elements of VREF isolates of animal and human origin

Source	Denmark			Norway			The Netherlands			England			United States			Finland			Saudi Arabia			France			Total		
	n	T	G	n	T	G	n	T	G	n	T	G	n	T	G	n	T	G	n	T	G	n	T	G	n	T	G
Humans	5	5	0	7	0	7 ^a	10	7	3	12	0	12	5	4	1				5	0	5	1	0	1	45	16	29
Pigs	30	29	1							3	3	0													33	32	1
Poultry	86	0	86	88	0	88	6	0	6	3	0	3				10	0	10							193	0	193
Total	121	34	87	95	0	95	16	7	9	18	3	15	5	4	1	10	0	10	5	0	5	1	0	1	271	48	223

^a All Norwegian human isolates were isolated from chicken farmers.

REFERENCES

1. 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.
2. Arthur, M., C. Molanas, F. Depardieu, and P. Courvalin. 1993. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J. Bacteriol.* **175**:117–127.
3. Bager, F., M. Madsen, J. Christensen, and F. M. Aarestrup. 1997. Occurrence of vancomycin resistant *Enterococcus faecium* in pig and poultry farms using avoparcin as a growth promotant. *Prev. Vet. Med.* **31**:95–112.
4. 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.
5. Jensen, L. B., P. Ahrens, L. Dons, R. N. Jones, A. M. Hammerum, and F. M. Aarestrup. 1998. Molecular analysis of Tn1546 in *Enterococcus faecium* isolated from animals and humans. *J. Clin. Microbiol.* **36**:437–442.
6. 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.
7. Klare, I., H. Heier, H. Claus, G. Böhme, 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 foodstuff and fecal samples of humans in the community. *Microb. Drug Resist.* **1**:265–272.
8. Schouten, M. A., A. Voss, and J. A. A. Hoogkamp-Korstanje. 1997. VRE and meat. *Lancet.* **349**:1258.
9. van den Bogaard, A. E., L. Bogø Jensen, and E. E. Stobberingh. 1997. Vancomycin-resistant enterococci in turkeys and farmers. *New Engl. J. Med.* **337**:1558–1559.
10. Wegener, H. C., M. Madsen, N. Nielsen, and F. M. Aarestrup. 1997. Isolation of vancomycin resistant *Enterococcus faecium* from food. *Int. J. Food Microbiol.* **34**:57–66.

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