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Vancomycin-Resistant Enterococci from Humans and Retail Chickens in Taiwan with Unique VanB Phenotype-*vanA* Genotype Incongruence

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Vancomycin resistant enterococci (VRE) with VanB phenotype-*vanA* genotype incongruence were found in all 39 VRE isolated from chicken carcasses and four human VRE isolates in Taiwan. Three identical mutations in the *vanS* gene were found in the VanB phenotype-*vanA* genotype VRE sequenced. This finding indicates possible transmission of glycopeptide resistance among different hosts.

VanA and VanB phenotypes of acquired glycopeptide resistance in enterococci have been well described (8, 21, 22). The VanA phenotype is defined as having high-level resistance to vancomycin and teicoplanin, whereas the VanB phenotype has various levels of vancomycin resistance and is not resistant to teicoplanin. Genes encoding the VanA- and VanB-phenotype resistance are located on transposons Tn*1546* and Tn*1547*, respectively, or in closely related transferable genetic elements, and the spread of vancomycin resistance gene clusters may be related to these transposable elements (2, 14, 25).

VanA-phenotype vancomycin resistant enterococci (VRE) have been recovered from farm animals, uncooked chickens, pork, and beef as well as feces of individuals living in the community in regions where avoparcin, a glycopeptide analog, has been used as a growth promoter of food animals (1, 4, 20, 28). Avoparcin was used in Taiwan for several years until January 2000, when it was banned from use as a feed additive. The first human VRE isolate (*Enterococcus faecalis*) from Taiwan was reported in 1996, and additional VRE cases were reported in 1999 (7, 9). These VRE isolates were resistant to vancomycin and susceptible to teicoplanin, consistent with the VanB phenotype, but reportedly possessed the *vanA* resistance gene; no explanation for the VanB phenotype-*van*A genotype incongruence was reported. However, a recent report by Hashimoto et al. suggests that mutations in the *vanS* regulatory gene can result in impaired resistance to teicoplanin among VRE isolates possessing the *vanA* gene cluster (15). Earlier data suggested deletions in *vanZ* could also result in a loss of teicoplanin resistance (3, 26).

The rate of vancomycin resistance among human enterococci in Taiwan is currently estimated at around 2%; both VanA and VanB phenotypes have been reported (9, 17). To determine whether VRE isolates are present in the food supply in Taiwan, we performed a survey of retail chickens sold in and around Taipei. We then compared the phenotypes and genotypes of chicken VRE isolates to human VRE isolates obtained from Taiwan hospitals.

(This study was presented in part at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, 17 to 20 September, 2000 [T.-L. Lauderdale, L. C. McDonald, Y. R. Shiau, P. C. Chen, H. Y. Wang, J. F. Lai, and M. Ho, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-1785, p. 107, 2000].)

Thirty chickens were purchased from nine different districts of the Taipei area, including six supermarkets (10 chickens) and 10 traditional markets (20 chickens). Fifteen chickens each were cultured in October 1999 and April 2000. Each whole chicken carcass was rinsed in 400 ml of buffered peptone water, which was centrifuged, and the pellet was used to inoculate bile esculin azide broth (Enterococcosel broth) containing aztreonam (50 μg/ml; kindly provided by Bristol-Myers Squibb Company, New York, N.Y.) and vancomycin (10 μg/ml; Sigma Chemicals, St. Louis, Mo.). Following incubation, 10 μl was subcultured to Columbia nalidixic acid agar plates, and colonies suspected to be *Enterococcus* spp. were identified using the Vitek GPI card (bioMérieux Vitek, Inc., Hazelwood, Mo.) and conventional biochemicals following standard protocols (13). Human VRE were from the Taiwan Surveillance of Antimicrobial Resistance and were collected in the fall of 1998 (16).

Susceptibilities to 10 antibiotics were determined by disk diffusion following the National Committee on Clinical Laboratory Standards (23). The MICs of teicoplanin were determined using the Etest according to the instructions of the manufacturer (AB BIODISK, Solna, Sweden). The vancomycin MIC was determined by agar dilution (24).

Total DNA was prepared using the DNeasy Tissue kit (Qiagen Inc., Valencia, Calif.) according to the manufacturer's instructions. All purified DNA was stored at −20°C until use. A multiplex PCR assay using primers specific for *vanA* and *vanB* resistance genes and the *E. faecalis* and *Enterococcus faecium* D-alanyl-D-alanine ligase genes was performed to determine the glycopeptide resistance genotype and to confirm the organism identification following published protocols (12). The PCRs were performed in a GeneAmp PCR system 9600

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TABLE 1. Glycopeptide resistance phenotypes and genotypes of chicken and human VRE isolates

VRE (no. of isolates)	Source	MIC (μg/ml) (interpretation[a]) of:		Phenotype	Genotype
		Vancomycin	Teicoplanin		
E. faecalis (27)	Chicken	≥1,024 (R)	0.75–8 (S)	VanB	*vanA*
E. faecium (12)	Chicken	≥1,024 (R)	0.25–1.0 (S)	VanB	*vanA*
E. faecalis (4)	Human	>1,024 (R)	2–8 (S)	VanB	*vanA*
E. faecalis (3)	Human	>1,024 (R)	12 (I)	VanB?	*vanA*
E. faecium (3)	Human	512–1,024 (R)	0.75–1.0 (S)	VanB	*vanB*
E. faecium (7)	Human	>1,024 (R)	24 (R)	VanA	*vanA*

[a] Interpretive criteria based on National Committee for Clinical Laboratory Standards. S, susceptible; I, intermediate; R, resistant. Interpretations for vancomycin: S, ≤4 μg/ml; I, 8 to 16 μg/ml; R, ≥32 μg/ml. Interpretations for teicoplanin: S, ≤8 μg/ml; I, 16 μg/ml; R, ≥32 μg/ml.

(Applied Biosystems, Foster City, Calif.). All PCR reagents, including primers, were from Gibco BRL (Life Technologies, Rockville, Md.).

To analyze the *vanS* and *vanZ* of the VanA-type determinants, primers specific for each gene were used for PCR (15). The PCR products were purified (Qiagen) and then sequenced using the Big Dye cycle sequencing ready reaction kit on an ABI PRISM 3700 DNA sequencer (Applied Biosystems). For homology analysis, the GenBank database was used. Control organisms used included *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, and *E. faecium* BM4147.

A total of 39 VRE, including 27 *E. faecalis* (from 26 chickens) and 12 *E. faecium* (from 12 chickens) isolates, were recovered from 28 of the 30 chickens. One chicken had two *E. faecalis* strains with different antibiograms. Ten of the 28 chickens had one each of *E. faecalis* and *E. faecium*. The 17 human isolates were from 17 individual patients.

The phenotypes and genotypes of all chicken and human VRE are presented in Table 1. All chicken VRE were highly resistant to vancomycin and susceptible to teicoplanin, consistent with the VanB phenotype. However, all these chicken VRE possessed the *vanA* genotype. Similarly, four human VRE (all *E. faecalis*) possessed the same VanB phenotype-*vanA* genotype incongruence. In addition, the other three human *E. faecalis* isolates were intermediate to teicoplanin yet possessed the *vanA* genotype.

The chicken and human VRE isolates with VanB phenotype-*vanA* genotype incongruence that had the *vanS* gene sequenced contained the three point mutations described by Hashimoto et al. (Table 2). In addition, one of the three human teicoplanin-intermediate *E. faecalis* possessed all three mutations in *vanS*; the other two had two of the three mutations in *vanS*. None of the three *vanS* point mutations were found in teicoplanin-resistant strains (Table 2). The *vanZ* sequences of all the *vanA* genotype tested (10 chicken and 14 human VRE) were identical to that reported to confer teicoplanin resistance (3), regardless of whether they were susceptible or resistant to teicoplanin (Table 2).

The predominance of *E. faecalis* strains possessing the VanB phenotype in the present study stands in contrast to reports from other countries where VRE recovered from either animals or the human food supply most commonly consist of *E. faecium* with the VanA phenotype (6, 11, 18, 27). To date, there have been very few reports of VRE with VanB phenotype-*vanA* genotype incongruence (15, 26). The finding of identical *vanZ* gene sequences in both VanA phenotype-*vanA* genotype and Van B phenotype-*vanA* genotype VRE suggests that the VanB phenotype-*vanA* genotype incongruity of our isolates was not due to mutations or deletions in the *vanZ* gene.

Hashimoto et al. reported that three point mutations located in the putative sensor domain of *vanS* are responsible for impaired teicoplanin resistance among *vanA*-genotype VRE (15). Our finding of VRE with VanB phenotype-*vanA* genotype incongruence with identical *vanS* point mutations in both human and retail chicken strains indicates possible transmission of glycopeptide resistance among different hosts. Pulsed-field gel electrophoresis performed on the first 13 chicken and the seven human VanB phenotype-*vanA* genotype VRE (*E. faecalis*) showed distinct populations; no clones common to both chicken and human isolates were found (data not shown). The fact that the VanB phenotype-*vanA* genotype VRE was found in all *E. faecalis* and *E. faecium* chicken isolates and that the human and chicken VRE were isolated from different time

TABLE 2. Nucleotide changes[a] in *vanS* of chicken and human VRE isolates

VRE (no. of isolates sequenced)	Source	Teicoplanin MIC (μg/ml) (interpretation[b])	Phenotype	Genotype	No. of isolates for which *vanZ* was sequenced[c]	Change at nucleotide:		
						4796 (T→G)	4808 (G→C)	4855 (A→T)
E. faecalis (19)	Chicken	0.75–8 (S)	VanB	*vanA*	7	Yes	Yes	Yes
E. faecium (11)	Chicken	0.25–1.0 (S)	VanB	*vanA*	3	Yes	Yes	Yes
E. faecalis (4)	Human	2–8 (S)	VanB	*vanA*	4	Yes	Yes	Yes
E. faecalis (1)	Human	12 (I)	VanB?	*vanA*	1	Yes	Yes	Yes
E. faecalis (2)	Human	12 (I)	VanB?	*vanA*	2	Yes	No	Yes
E. faecium (7)	Human	24 (R)	VanA	*vanA*	7	No	No	No

[a] As reported by Hashimoto et al. (15).
[b] See Table 1 footnote *a*.
[c] *vanZ* sequences of these isolates were all identical to the *vanZ* of Tn*1546*.

periods also indicate that these isolates were not from a common, widespread clone. Several studies have suggested that horizontal gene transfer may be more important than clonal dissemination of vancomycin resistance (11, 12, 14, 26, 27). Reports from other countries also suggest colonization of chickens with VRE is related to the use of avoparcin as a growth promoter (1, 4, 5, 10, 19). Avoparcin has been banned from use as a feed additive in Taiwan. We will continue to monitor and characterize VRE for changes in resistance development and evidence of resistance transfer among different hosts.

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