APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2003, p. 7414–7419 Vol. 69, No. 12
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.12.7414–7419.2003

Predominance of *vanA* Genotype among Vancomycin-Resistant *Enterococcus* Isolates from Poultry and Swine in Costa Rica

Warner Bustamante,[1] Angélica Alpízar,[1] Silvia Hernández,[1] Alexandra Pacheco,[1]† Natalia Vargas,[1] Marco Luis Herrera,[2] Álvaro Vargas,[2] Magaly Caballero,[3] and Fernando García[1]*

Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica,[1] and Hospital Nacional de Niños,[2] San José, and Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia,[3] Costa Rica

Received 2 April 2003/Accepted 10 September 2003

The use of avoparcin as a growth promoter is considered to have selected for vancomycin-resistant enterococci (VRE). In Costa Rica, the use of avoparcin for poultry and swine was intensive until the product was withdrawn from the market in 2000. We evaluated the presence of VRE in poultry, swine, and cattle fecal samples obtained during 1998 and 1999. A total of 185 VRE isolates were recovered from 116 out of 893 samples. *Enterococcus faecium* was the most frequently isolated species (50.8%), being predominant among poultry (71.6%) and swine (37.7%) isolates, but it was not recovered from the bovine samples. The second-most-frequently-isolated species from poultry and swine, respectively, were *E. durans* (23.2%) and *E. faecalis* (21.7%). *E. casseliflavus* was the only species obtained from bovine samples, but it was not found among the avian isolates. An evident predominance of the *vanA* determinant among vancomycin-resistant enterococcal species from poultry and swine, but not from cattle, was observed and was similar to the situation in European countries before avoparcin was forbidden. The diversity of the *vanA* determinant in the isolates was assessed by detection of the IS*1251* insertion in the *vanSH* intergenic region and of the IS*1476* insertion in the *vanXY* intergenic region. However, in none of the 154 *vanA*$^+$ isolates recovered in this study were those insertions detected.

Enterococcus spp. are gram-positive, catalase-negative cocci that are considered a dominant bacterial group in the intestinal flora of humans and animals (18). They are involved in community-acquired and nosocomial infections such as urinary tract infections, bacteremia, endocarditis, and others. There has been an increased interest in these bacteria because of their resistance to multiple antimicrobial drugs, including both inherent resistance to penicillins, cephalosporins, clindamycin, and aminoglycosides and plasmid- and transposon-mediated resistance to tetracyclines, macrolides, and high levels of trimethoprim, clindamycin, aminoglycosides, and glycopeptides (25, 31).

Resistance to vancomycin and other glycopeptides is heterogeneous. Three phenotypes, VanA, VanB, and VanC, have been well described (9, 38), but three additional phenotypes, VanD, VanE, and VanG, have recently been reported (19, 22, 30, 34). Both *vanA* and *vanB* determinants are transferable by conjugation among enterococci, including *E. faecalis*, *E. faecium*, *E. avium*, and *E. raffinosus* (9, 20, 38). The *vanA* gene cluster is located on a mobile DNA element, Tn*1546*, and consists of seven genes denominated *vanR*, *vanS*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ* (9, 38). The heterogeneity of Tn*1546* has previously been reported (39), including the insertion of IS*1251* in the *vanSH* intergenic region (24) and of IS*1476* in the *vanXY* intergenic region (5). A similar gene structure has been described for the *vanB* determinant located on Tn*1547* in *E. faecalis* V583 (20). In contrast, *vanC1*, *vanC2*, and *vanC3* are normally occurring genes present in *E. gallinarum*, *E. casseliflavus*, and *E. flavescens*, respectively, and are considered not to be transferable (9, 38).

Supplementing animal food with antimicrobial agents to enhance growth has been a common practice for more than 30 years. This usage is estimated to constitute more than half the total antimicrobials used worldwide. Previous studies have shown that the use of antimicrobial growth promoters may select resistant bacteria among the normal intestinal flora of animals that can be transmitted to humans (2, 3, 6). Several studies in European countries indicate that the use of avoparcin as a growth promoter has contributed to the creation of a major reservoir of several species of *Enterococcus* carrying the *vanA* determinant in food animals and that vancomycin-resistant enterococci (VRE) can be transmitted from animals to humans (9). Avoparcin has never been approved for use in the United States by federal authorities. Although several studies have been conducted in industrialized countries of VRE in hospitals, communities, and food animals, little is known about the epidemiology and dissemination of VRE in developing countries. Notwithstanding the experiences in the United States and Europe, the use of avoparcin for growth promotion in poultry and pigs was unrestricted in Costa Rica until Roche voluntary withdrew avoparcin from the market by the first quarter of 2000. This study was undertaken to determine the occurrence of VRE in food animal settings in Costa Rica during 1998 and 1999, prior to the withdrawal of avoparcin

* Corresponding author. Mailing address: Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, 2060 Ciudad Universitaria Rodrigo Facio, San José, Costa Rica. Phone: (506) 207 4275. Fax: (506) 225 2374. E-mail: fgarcia@cariari.ucr.ac.cr.

† Present address: Instituto Costarricense de Acueductos y Alcantarillados, Puntarenas, Costa Rica.

TABLE 1. Isolation of VRE from animal fecal samples in Costa Rica

Source	No. of samples	No. (%) positive for VRE
Poultry	413	61 (14.8)
Sampling 1	146	21 (14.4)
Sampling 2	119	9 (7.6)
Sampling 3	148	31 (21.0)
Swine	350	38 (10.9)
Farm 1	100	3 (3.0)
Farm 2	100	11 (11.0)
Farm 3	50	7 (14.0)
Farm 4	100	17 (17.0)
Cattle	130	17 (13.1)
Farm 5	10	2 (20.0)
Farm 6	60	0 (0.0)
Farm 7	60	15 (25.0)
Total	893	116 (13.0)

from the market. The results presented in this paper show that the *vanA* determinant is predominant among enterococcal species from poultry and pigs, but not from cattle, a situation similar to that in European countries before the use of avoparcin was forbidden, and that food animals may represent an important reservoir of VRE in Costa Rica.

MATERIALS AND METHODS

Reference strains. The following *Enterococcus* strains were used in this study as controls for identification tests, determination of MIC, and PCR analysis: *E. faecium* A256 (*vanA*$^+$), *E. faecalis* V583 (*vanB*$^+$), *E. gallinarum* VR42 (*vanC-1*$^+$), and *E. casseliflavus* ATCC 25988 (*vanC-2*$^+$) (11). The vancomycin-susceptible strain *E. faecalis* ATCC 29212 was also used.

Fecal samples. For broilers, a total of 413 cloacal samples were collected by using swabs during three different visits to a national poultry slaughterhouse receiving chickens from all around the country. The chickens were on average 5 to 6 weeks old. In addition, 130 cecal samples were collected from adult dairy cows at three different farms. A total of 350 cecal samples were collected from swine at four different farms by using rectal swabs (Table 1). Swine fecal samples were collected from newly weaned (5 to 10 kg), growing (20 to 60 kg), and finishing (60 to 100 kg) pigs, as well as from gestating and lactating sows. An equal number of samples were collected from each group of pigs at a particular farm. In all samplings, animals were randomly selected from the entire flock or herd. The number of samples collected from cattle and swine represented approximately 5% in each herd, whereas those from poultry represented approximately 2.5% of each flock. Samples from poultry were collected during 1998, whereas samples from pigs and cattle were collected during 1999. Precise information about avoparcin use in the poultry flocks and swine herds sampled for this study was not available. However, avoparcin was not used for the three bovine herds sampled.

Isolation and identification of VRE from fecal samples. Fecal samples were analyzed for the presence of VRE by enrichment of the samples in buffered peptone broth (10 g of peptone/liter, 5 g of NaCl/liter, 3.5 g of Na_2HPO_4/liter, and 1.5 g of KH_2PO_4/liter, pH 7.2) for 18 h at 35°C and selection in brain heart infusion (Oxoid Ltd., Basingstoke, Hampshire, England) agar plates containing 6 μg of vancomycin/ml (Sigma Chemical Co., St. Louis, Mo.) and 0.04% (wt/vol) NaN_3 (Sigma) for 24 to 48 h at 35°C. A maximum of five colonies were selected from each agar plate according to the size, morphology, and pigmentation of the colonies. When no obvious variations were observed, at most three colonies were randomly selected from each agar plate. Preliminary identification of the isolates was performed by conventional biochemical tests (gram-positive cocci, catalase negative, growth at 45°C, growth in 6.5% NaCl, and bile-esculin positive). Isolates showing typical enterococci were identified by using the Vitek system (bioMérieux, Marcy l'Etoile, France) with the GPI cards according to the instructions of the manufacturer. Additional biochemical tests were performed as previously described to confirm speciation, including production of acid in the presence of arabinose, mannitol, raffinose, sorbitol, sorbose, and sucrose; utilization of pyruvate; resistance to 0.04% (wt/vol) tellurite; production of pigment; and motility (18). MIC and PCR analyses (see below) were performed only for those isolates from each fecal sample showing different biochemical patterns.

Determination of MIC. The MIC of vancomycin (Sigma) was determined by the agar dilution method approved by the NCCLS (32). Each isolate was grown on blood agar plates without antibiotics for 18 h at 35°C and suspended in sterile saline solution to a density of 0.5 MacFarland standard. A 1:100 dilution from each suspension was prepared in sterile saline solution. Ten microliters from each dilution, containing approximately 10^4 CFU per ml, was spotted on brain heart infusion agar plates containing different concentrations of vancomycin. The inoculated plates were incubated in the dark for 24 h at 35°C.

Other tests. β-Lactamase production and high-level aminoglycoside resistance (HLR) to gentamicin and streptomycin were assessed as described previously (26, 27).

DNA isolation and PCR analysis of *van* genotypes. DNA was isolated by using a previously described procedure (21) and was stored at −20°C until use. The *van* genotypes were determined by PCR analysis by using the following primers (21): vanA-F, 5′-GCTATTCAGCTGTACTC-3′; vanA-R, 5′-CAGCGGCCATCATACGG-3′; vanB-F, 5′-CATCGCCGTCCCCGAATTTCAAA-3′; vanB-R, 5′-GATGCGGAAGATACCGTGGCT-3′; vanC1-F, 5′-GGTATCAAGGAAACCTC-3′; vanC1-R, 5′-CTTCCGGCCATCATAGCT-3′; vanC2-F, 5′-CTCCTACGAATTCTCTTG-3′; and vanC2-R, 5′-CGAGCAAGACCTTTAAG-3′. Amplification reactions were performed in a solution containing 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, and 0.05% (wt/vol) Tween 20 supplemented with 100 μM (each) deoxynucleoside triphosphate, 0.4 μM (each) primer, and 1.5 U of recombinant *Taq* DNA polymerase (AmpliTaq; Roche Molecular Systems, Inc., Branchburg, N.J.) in a final volume of 50 μl. Each sample was subjected to 30 cycles, each consisting of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C, with a final extension at 72°C for 5 min in a GeneAmp PCR System 2400 (Perkin-Elmer Corporation, Norwalk, Conn.). Amplification products were visualized on 1.5% agarose gels stained with ethidium bromide. The sizes of the amplification and digestion products were estimated by comparison with DNA size markers (123-bp ladder; Sigma). All PCR analyses were performed in duplicate. In order to analyze the heterogeneity of the *vanA* determinant, the *vanSH* and *vanXY* intergenic regions were amplified by PCR as previously described (29). According to this procedure, amplification products of 2,337 and 1,947 bp, respectively, were expected when no insertion sequence elements were inserted in the *vanSH* and *vanXY* intergenic regions. Amplification products of a higher size are expected when insertion sequence elements are inserted in these intergenic regions.

RESULTS

Isolation and identification of VRE from fecal samples. A total of 893 fecal samples were collected from poultry (n = 413), swine (n = 350), and cattle (n = 130), as indicated in Table 1. In order to detect diversity among the VRE isolates, a maximum of five colonies were selected from each sample for further processing. Three isolates, two from poultry samples and one from a swine sample, were initially considered enterococci, but their identification by the Vitek system could not be confirmed. Additional tests indicated that these three isolates belong to the genus *Leuconostoc*, and they were not further analyzed. Vancomycin-resistant *Enterococcus* species and *Streptococcus equinus* were recovered from 116 (13%) of the samples. The isolation rates for the different samplings vary from 0 to 25.0%. As shown in Table 2, only one biochemically distinguishable VRE isolate was detected in 63 (54.3%) of the positive samples. In 53 (45.7%) of the positive samples, two to four biochemically distinguishable VRE isolates were detected. These samples included seven poultry samples and three pig samples from which two different species of VRE were recovered. In these poultry samples, *E. faecium* and *E. durans* were detected in three samples, *E. faecium* and *E. gallinarum* were detected in two samples, and *E. faecium* and

TABLE 2. Isolation of VRE from poultry, swine, and cattle fecal samples in Costa Rica

Source	No. of isolates per sample[a]				No. of positive samples	No. of VRE isolates
	1	2	3	4		
Poultry	32	24	5		61	95
Swine	18	11	7	2	38	69
Cattle	13	4			17	21
Total	63	39	12	2	116	185

[a] Number of distinguishable biochemical patterns for isolates recovered from the same samples.

E. faecalis were detected in two samples, whereas the species *E. casseliflavus* and *E. gallinarum*, *E. faecium* and *E. hirae*, and *E. faecium* and *E. faecalis* were detected in each of these three swine samples. In 22 samples from poultry, 15 from swine, and 4 from cattle, two or three isolates belonging to the same species but showing different biochemical patterns were obtained. In each of two swine samples, four biochemically distinguishable isolates of *E. casseliflavus* and *E. faecium*, respectively, were detected.

Ninety-five isolates were recovered from the 61 poultry samples, 69 isolates were recovered from the 38 swine samples, and 21 isolates were recovered from the 17 bovine samples (Table 2). Thus, a total of 185 vancomycin-resistant isolates were recovered from the 116 fecal samples. The results of the identification of the animal VRE isolates are shown in Table 3. *E. faecium* was the most frequently isolated species (50.8%), being predominant among the poultry (71.6%) and swine (37.7%) isolates, but it was not recovered from the bovine samples. *E. casseliflavus* was the only species obtained from bovine samples, but it was not found among the avian isolates. The second-most-frequently-isolated species from poultry and swine, respectively, were *E. durans* (23.2%) and *E. faecalis* (21.7%). However, *E. durans* was not identified among the swine and bovine isolates. *S. equinus* showing resistance to vancomycin was isolated from one fecal sample from poultry and one from swine.

MICs of vancomycin and *van* genotypes. The MICs of vancomycin were >512 μg/ml for 91.5%, 256 μg/ml for 5.3%, and 128 μg/ml for 3.2% of the isolates recovered from poultry. For 59 out of 69 (85.5%) swine isolates, the MIC of vancomycin was >512 μg/ml, whereas for the rest of the 10 swine isolates, including two *E. casseliflavus* and eight *E. gallinarum* isolates, it was ≤16 μg/ml. The MIC of vancomycin for all *E. casseliflavus* isolates recovered from cattle was 8 μg/ml. The two *S. equinus* isolates analyzed in this study required MICs of vancomycin of >512 μg/ml. The MICs of vancomycin at which 50% of the strains are inhibited (MIC_{50}s) and the MIC_{90}s for the VRE isolates are shown in Table 4. The presence of the different *van* determinants was assessed by PCR. The *vanA* determinant was observed among various enterococcal species, including *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. hirae* (Table 5). The *vanA* determinant was also observed in the two *S. equinus* isolates recovered in this study. The *vanA* genotype was detected in all isolates obtained from poultry samples with a MIC of vancomycin of ≥128 μg/ml and in all swine isolates with a MIC of ≥512 μg/ml but was not detected among vancomycin-resistant *E. casseliflavus* isolates of bovine origin. The *vanA* determinant was found in all *E. faecium* isolates recovered in this study (Table 5). The diversity of the *vanA* determinant of the isolates was assessed by the detection of insertions of IS*1251* in the *vanSH* intergenic region and of IS*1476* in the *vanXY* intergenic region. However, in none of the 154 $vanA^+$ isolates recovered in this study were these insertions detected (data not shown), since all amplification product sizes from the *vanSH* and *vanXY* intergenic regions of these isolates were approximately 2,340 and 1,950 bp, respectively. Amplification products of similar size were also detected in *E. faecium* strain A256. The *vanC* determinant was detected in 10 out of 69 (14.5%) isolates from swine samples, including two *E. casseliflavus* (*vanC-2*) and eight *E. gallinarum* isolates (*vanC-1*), and in all *E. casseliflavus* (*vanC-2*) isolates from bovine samples (Table 5). Interestingly, the *vanB* genotype was not observed among the 185 isolates analyzed in this study.

TABLE 3. Identification of VRE isolates from poultry, swine, and bovine fecal samples

Species	No. (%) of isolates found in samples from:			Total no. (%) of isolates
	Poultry	Swine	Cattle	
E. casseliflavus	0 (0.0)	10 (14.5)	21 (100.0)	31 (16.7)
E. durans	22 (23.2)	0 (0.0)	0 (0.0)	22 (11.9)
E. faecalis	2 (2.1)	15 (21.7)	0 (0.0)	17 (9.2)
E. faecium	68 (71.6)	26 (37.7)	0 (0.0)	94 (50.8)
E. gallinarum	2 (2.1)	10 (14.5)	0 (0.0)	12 (6.5)
E. hirae	0 (0.0)	7 (10.1)	0 (0.0)	7 (3.8)
S. equinus	1 (1.0)	1 (1.5)	0 (0.0)	2 (1.1)
Total	95 (100.0)	69 (100.0)	21 (100.0)	185 (100.0)

TABLE 4. MICs of vancomycin and percentage of VRE *van* genotypes isolated from fecal samples from poultry, swine, and cattle that were *vanA* or *vanC*

Source	Vancomycin MIC (μg/ml)		% *vanA*	% *vanC*
	MIC_{50}	MIC_{90}		
Poultry	>512	>512	100.0	0.0
Swine	>512	>512	85.5	14.5
Cattle	8	8	0.0	100.0

β-Lactamase production and HLR phenotype. β-Lactamase activity was detected in eight $vanA^+$ isolates (4.3%), including one *E. gallinarum* isolate and five *E. faecium* isolates from poultry and one *E. faecalis* isolate and one *E. hirae* isolate from swine. The HLR phenotype was assessed for resistance to streptomycin and gentamicin. The HLR phenotype for streptomycin resistance was observed in 110 out of 185 (59.5%) isolates, all carrying the *vanA* determinant. Forty-four isolates (23.8%) carrying the $vanA^+$ determinant and 31 isolates lacking *vanA* (16.7%) were susceptible to streptomycin. Only one *E. durans* $vanA^+$ isolate recovered from poultry showed the HLR phenotype for both gentamicin and streptomycin resistance, but no β-lactamase activity was detected in this isolate.

TABLE 5. Distribution of *van* determinants among enterococcal species of animal origin

Species	Poultry			Swine			Cattle			Total no. of isolates	No. (%) of *vanA*$^+$ isolates	No. (%) of *vanC*$^+$ isolates
	No. of isolates	No. of *vanA* isolates	No. of *vanC*[a] isolates	No. of isolates	No. of *vanA* isolates	No. of *vanC*[a] isolates	No. of isolates	No. of *vanA* isolates	No. of *vanC*[a] isolates			
E. casseliflavus	0	0	0	10	8	2	21	0	21	31	8 (23.3)	23 (76.7)
E. durans	22	22	0	0	0	0	0	0	0	22	22 (100.0)	0 (0.0)
E. faecalis	2	2	0	15	15	0	0	0	0	17	17 (100.0)	0 (0.0)
E. faecium	68	68	0	26	26	0	0	0	0	94	94 (100.0)	0 (0.0)
E. gallinarum	2	2	0	10	2	8	0	0	0	12	4 (33.3)	8 (66.7)
E. hirae	0	0	0	7	7	0	0	0	0	7	7 (100.0)	0 (0.0)
S. equinus	1	1	0	1	1	0	0	0	0	2	2 (100.0)	0 (0.0)
Total	95	95	0	69	59	10	21	0	0	185	154 (83.2)	31 (16.8)

[a] *vanC-1* in *E. gallinarum*; *vanC-2* in *E. casseliflavus*.

DISCUSSION

In this study, we report the isolation of VRE from feed animals in Costa Rica. VRE were recovered from fecal samples obtained from swine, poultry, and cattle. In almost half of the positive samples, two to four biochemically distinguishable VRE isolates were detected, indicating that *van* determinants are distributed among diverse enterococcal clones or species in a single animal. In fact, in nine samples, six from poultry and three from swine, two different species of VRE carrying the *vanA* determinant were detected. In 44 of these samples, two to four isolates belonging to the same enterococcal species but showing different biochemical profiles were recovered. All isolates recovered from the same animal carried the same *van* determinant.

E. faecium was the most frequently isolated VRE species, being predominant among the poultry and swine isolates, but it was not recovered from the bovine samples. *E. casseliflavus* was the only species obtained from bovine samples, but it was not found among the avian isolates. The second-most-frequently-isolated species from poultry and swine, respectively, were *E. durans* and *E. faecalis*. However, *E. durans* was not identified among the swine and bovine isolates. The distribution of the enterococcal species found among the different animal species correlates with previous reports, but some differences were observed (13–17, 28). In fact, *E. faecium* is the predominant species reported in fecal samples collected from poultry and swine, whereas *E. hirae*, a species recovered in this study only from swine samples, has been reported as a species frequently present in the feces of poultry (14, 28). In addition, it has been reported that in the feces of adult dairy cows there are hardly any enterococcal species present and that the main part of the flora consists of *Streptococcus bovis* (15, 17). However, in this study, we recovered *E. casseliflavus vanC-2* isolates from the fecal bovine samples. Finally, *E. cecorum*, reported to be a predominant species in fecal samples from ruminant calves and pigs (14, 15, 16), was not recovered in the samples analyzed in this study, since this species requires CO_2 for growth and does not grow on enterococcus-selective media. However, to our knowledge, no isolate of *E. cecorum* has been reported to show resistance to glycopeptides or to carry *van* determinants. Thus, the differences observed in the species recovered from the animals could be due to the isolation method.

The *vanA* determinant was detected among various enterococcal species, including *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. hirae*, as well as in *S. equinus*, supporting the evidence that the *vanA* gene cluster located on mobile elements is able to disseminate between different species. The *vanA* determinant was detected mostly in isolates of poultry and swine origin, but it was absent among the bovine isolates. All isolates carrying the *vanA* determinant showed MICs of vancomycin of $\geq$128 μg/ml, and most of the *vanA*$^+$ isolates from swine showed MICs of vancomycin of $\geq$512 μg/ml. These data correlate well with the use of avoparcin in Costa Rica as a growth promoter in poultry and swine but not in cattle. Thus, whereas most of these species, with the exceptions of *E. faecalis* and *E. faecium*, are considered of reduced virulence for animals and humans, they constitute an important reservoir for the *vanA* determinant that can eventually be transmitted to enterococcal strains colonizing the human gas-

trointestinal and genitourinary tracts. The *vanC* determinant was detected in eight *E. gallinarum* (*vanC-1*) and two *E. casseliflavus* (*vanC-2*) isolates recovered from swine samples and in all *E. casseliflavus* (*vanC-2*) isolates from bovine samples. The *vanB* determinant was not observed among the enterococcal isolates recovered in this study. As has previously been reported, *vanA*-type resistance is widely distributed and is by far the predominant type of resistance reported in Europe. While *vanB*-type resistance is fairly common among enterococcal isolates in the United States, *vanA*-type resistance still predominates (10). In addition, the *vanA* gene cluster has been found among other gram-positive bacteria, including the genera *Streptococcus*, *Lactococcus*, *Oerskovia*, *Arcanobacterium*, and *Bacillus* (9, 20, 38), and it can be transferred by conjugation under laboratory conditions to *Streptococcus pyogenes*, viridans group streptococci, *Listeria monocytogenes*, and *Staphylococcus aureus* (12, 33). In contrast, the *vanB* determinant has been found primarily in *E. faecium* and *E. faecalis* (9, 20, 38), although a *vanB*-related gene sequence has been found in *Streptococcus bovis* (35). The difference in the dissemination of these determinants may be related to the observation that the *vanA* gene cluster is often located on a Tn*1546*-like transposon, which can be part of a conjugative plasmid (5, 9, 22, 23). Although the *vanB* gene cluster may be carried on a plasmid, it is usually on the chromosome, and its dissemination can occur by the transfer of large genetic elements which contain the transposon Tn*1547* (36, 37).

The heterogeneity of Tn*1546* can be useful for epidemiological studies (39). In this work, the heterogeneity of the *vanA* gene cluster was assessed by the presence of insertions of IS*1251* in the *vanSH* intergenic region (24) and of IS*1476* in the *vanXY* intergenic region (5). However, these insertions were not detected among the 154 *vanA*$^+$ isolates analyzed in this study or in *vanA*$^+$ reference strain *E. faecium* A256. These results suggest either predominance of a single clone or a low discriminatory power for this typing strategy. Additional studies are presently being undertaken in our laboratory to characterize the *vanA* gene cluster in the animal isolates.

Two additional traits, β-lactamase activity and HLR phenotype, were assessed among VRE isolates. β-Lactamase was detected in only eight *vanA*$^+$ isolates (4.3%), including six isolates from poultry and two isolates from swine. The HLR phenotype for streptomycin resistance was detected in 110 isolates (59.5%), all carrying the *vanA* determinant, whereas susceptibility to streptomycin was observed in 44 *vanA*$^+$ isolates (23.8%) and in 31 isolates lacking *vanA* (16.7%). A single poultry *E. durans vanA*$^+$ isolate showed the HLR phenotype for both gentamicin and streptomycin resistance, but no β-lactamase activity was detected in this isolate. Thus, while β-lactamase production and the HLR phenotype for gentamicin resistance are infrequent characteristics in VRE isolates analyzed in the study, the HLR phenotype for streptomycin resistance is a rather common trait among animal *vanA*$^+$ VRE.

The use of subtherapeutic levels of antibiotics in animal husbandry as growth promoters constitutes a selective pressure for the prevalence and dissemination of resistance determinants among bacterial populations. Avoparcin, a glycopeptide with a chemical structure which is very similar to that of vancomycin or teicoplanin, had been widely used in several European Union and developing countries for animal growth promotion but had never been approved for use in United States. Due to its association with a high prevalence of VRE in different settings, the use of avoparcin was banned in all European Union countries in April 1997. In spite of the experiences in the United States and Europe, no ban has been imposed in Costa Rica on the use of avoparcin and other antibiotics for animal growth promotion. In fact, avoparcin was used in Costa Rica for several years, until the first quarter of 2000, when it was no longer available on the market. According to the register of the Ministry of Agriculture of Costa Rica, during 1999, 15,000 kg of Avotan100, containing 100 g of avoparcin in each kg of product, was imported to Costa Rica. Once avoparcin was withdrawn, a reduction in the prevalence and dissemination of VRE similar to that described by several studies performed in Denmark, Germany, The Netherlands, and Italy was expected. However, the ban of avoparcin as a growth promoter could not be immediately translated to a reduction in the prevalence of VRE in food animals. In fact, although the use of avoparcin was banned in Denmark in 1995, data from the Danish antimicrobial resistance monitoring program showed a marked reduction in VRE from broilers, but no significant change occurred in pigs (4, 7, 8). The persistence of VRE in pigs could be attributed to coselection resulting from continued use of tylosin, because genes encoding resistance to macrolides and glycopeptides are linked among isolates from pigs (1). Thus, genetic linkage and coselection could weaken the impact of the restrictive use of antibiotics on the prevalence and dissemination of antimicrobial resistance determinants in bacterial populations.

In conclusion, the *vanA* determinant is predominant among diverse enterococcal species from poultry and pigs but not from cattle, a situation similar to that in European countries before the use of avoparcin was banned. Food animals may represent an important reservoir for VRE in Costa Rica and appear to play an important role in the prevalence, acquisition, and dissemination of antibiotic resistance determinants.

ACKNOWLEDGMENTS

This work was financially supported by research grants from the Universidad de Costa Rica and the Universidad Nacional.

We gratefully acknowledge D. Utzinger for her technical assistance, G. Bonilla and J. R. Molina for their assistance in the collection of the swine samples, and H. Raventós for critical reading of the manuscript.

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