

## Presence of Vancomycin-Resistant Enterococci in Farm and Pet Animals

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***Enterococcus faecium* strains with *vanA*-mediated glycopeptide resistance were isolated by enrichment culture from the intestines and feces of several animal species, mainly horses and dogs (8% positive), chickens (7% positive), and pigs (6% positive). Other *vanA*-positive enterococcal strains were identified as *E. durans* in gallinaceous birds, *E. faecalis* in a horse, and *E. gallinarum* in a pheasant. Samples from pigeons, cage birds, and ruminants were negative. It was concluded that vancomycin resistance is widespread among isolates from farm and pet animals.**

Vancomycin (glycopeptide)-resistant enterococci (VRE) may cause serious problems in hospitalized patients. Until recently, the possible presence of such strains in animals was unknown and remained uninvestigated, probably because glycopeptide antibiotics are not used therapeutically in veterinary medicine. In Europe, one member of this antibiotic group, avoparcin, is used for growth promotion in farm animals. *vanA*-carrying vancomycin-resistant *Enterococcus faecium* strains are cross-resistant to avoparcin (12). In a report on the in vitro susceptibility of animal enterococci isolated in 1979 to growth-promoting antibiotics (7), all strains investigated were found to be susceptible to avoparcin. In recent years, however, with the finding of VRE in wastewater of sewage treatment plants (2, 13, 16), feces of farm animals (1, 2, 12), meat (2, 11, 12), feces of nonhospitalized persons in Europe and the United Kingdom (10, 17), and in hospitals without vancomycin-resistant infections (8), interest in the possible role of animals as a source of vancomycin-resistant strains increased. In investigations carried out in Germany (11, 12) and Denmark (1), the occurrence of vancomycin resistance was linked to the use of avoparcin as a growth-enhancing antibiotic in animals.

In view of a possible involvement of animals in the spread of VRE, we undertook a broad survey of the presence of such strains in farm and pet animals.

### MATERIALS AND METHODS

**Samples.** The samples examined were collected in 1995 from animals originating from 557 different farms or owners in Belgium (Table 1). Fecal samples from 63 pigs, 50 horses, 12 rabbits, 6 parrots and parakeets, and 3 pigeons, litter samples from 35 poultry farms, and anal swabs from 14 cats and 9 dogs were cultured within 1 or 2 days after collection. The remaining samples were taken from the intestines of animals sent in for postmortem diagnosis. These were stored for up to 3 weeks at  $-20^{\circ}\text{C}$  before examination. Except for the poultry litter, all samples were collected from individual animals, and only samples from one animal per farm or per owner were examined.

**Enrichment culture of VRE.** Samples of 0.5 to 1 g were inoculated in 5 or 10 ml of kanamycin esculin azide broth (Lab M, Bury, United Kingdom) supplemented with 20  $\mu\text{g}$  of vancomycin per ml, as described by Bates et al. (2). Tubes whose contents turned black after 1 or 2 days of incubation at  $37^{\circ}\text{C}$  were subcultured onto kanamycin esculin azide agar (Lab M) to which 20  $\mu\text{g}$  of vancomycin per ml was added.

**Identification.** Suspected enterococcal colonies were purified on Slanetz and Bartley agar (Oxoid, Basingstoke, United Kingdom) and were identified by growth and biochemical reactions as described previously (4). The species identification of vancomycin-resistant strains was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) analysis of whole-cell proteins. The strains were grown on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) for 24 h at  $37^{\circ}\text{C}$  in an atmosphere of 5%  $\text{O}_2$ , 10%  $\text{CO}_2$ , and 85%  $\text{N}_2$ . PAGE of whole-cell proteins, densitometric analysis, normalization and interpretation of protein profiles, and numerical analysis were performed as described previously (14). The strains were identified by using a database comprising the protein patterns of more than 700 enterococcal strains representing all known species.

**Susceptibility testing.** The vancomycin resistance of suspected colonies appearing on vancomycin-supplemented media was first confirmed by subculturing the colonies on sectorized Slanetz and Bartley agar (Oxoid) plates containing 20  $\mu\text{g}$  of vancomycin per ml. Isolates with enterococcus-like appearances growing on this medium were tested by the agar dilution procedure to determine the MICs of avoparcin and vancomycin. Tests were carried out on Mueller-Hinton II agar (BBL, Cockeysville, Md.) inoculated with standardized inocula prepared from overnight brain heart infusion (Oxoid) cultures, delivering approximately  $10^4$  CFU per spot.

**Detection of *vanA*, *vanB*, *vanC1*, and *vanC2* by PCR.** Detection of *vanA*, *vanB*, *vanC1*, and *vanC2* by PCR was attempted for all enterococcal strains isolated by vancomycin enrichment. For amplification of *vanA*, *vanB*, and *vanC1* genes, the PCR described by Clark et al. (3) and modified as follows was used: 5 and 10 bacterial colonies from a blood agar plate incubated overnight were suspended in a 50- $\mu\text{l}$  reaction mixture containing 0.1  $\mu\text{M}$  (each) primer and 0.5 U of *Taq* polymerase (Goldstar; Eurogentec, Seraing, Belgium). A Techne model PHC-2 DNA thermocycler was used and was programmed as follows: lysis and denaturation for 2 min at  $94^{\circ}\text{C}$ , hybridization for 1 min at  $58^{\circ}\text{C}$ , and elongation for 1 min at  $72^{\circ}\text{C}$  for 30 cycles; a first lysis and denaturation step of 2 min at  $95^{\circ}\text{C}$ ; and a final elongation step of 10 min at  $72^{\circ}\text{C}$ .

For the amplification of the *vanC2* gene, the oligonucleotide primers of Dutka-Malen et al. (6) were used in the same PCR mixture described above, with the following cycling:  $94^{\circ}\text{C}$  for 2 min for the first cycle,  $94^{\circ}\text{C}$  for 1 min,  $54^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 1 min for the next 30 cycles; the amplification was concluded with a cycle of  $72^{\circ}\text{C}$  for 10 min. The amplicons were revealed by electrophoresis on a 0.2% agarose gel (Hispanagar, Burgos, Spain).

### RESULTS

Thirty glycopeptide-resistant strains were isolated from samples from pigs, chickens, horses, dogs, pheasants, a cat, a rabbit, and a duck. The MICs of vancomycin were 256  $\mu\text{g}/\text{ml}$  or higher. The avoparcin MICs ranged from 64 to 256  $\mu\text{g}/\text{ml}$ . All samples from ruminants, pigeons, cage birds, ostriches, and diverse zoo animals were negative. Twenty-five of the resistant strains were identified as *E. faecium* and were found in 8% of the equine and canine samples, 7% of the poultry samples, and 6% of the pig samples. Two strains from chickens and one from a pheasant were identified as *E. durans*, one strain from

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TABLE 1. Origin and number of samples examined by an enrichment technique for the detection of VRE and number and origin of *vanA*-positive enterococcal strains isolated

Animal species	No. of samples examined	No. of <i>vanA</i> -positive strains isolated
Pigs	85	5
Chickens	80	8
Horses	83	8
Rabbits	33	1
Dogs	49	4
Cats	21	1
Ostriches	9	0
Zoo animals	10	0
Ruminants		
Cattle	55	0
Sheep	7	0
Deer	8	0
Goats	1	0
Cage and aviary birds		
Pigeons	34	0
Parrots and parakeets	32	0
Canaries	10	0
Finches	5	0
Pheasants	14	2
Anseriformes		
Swans	7	0
Ducks	13	1
Goose	1	0
Total	557	30

a horse was identified as *E. faecalis*, and another strain from a pheasant was identified as *E. gallinarum*.

All glycopeptide-resistant strains carried the *vanA* gene. The single *E. gallinarum* strain from a pheasant also possessed the *vanC1* gene.

## DISCUSSION

No glycopeptide resistance was found among enterococcal strains isolated on vancomycin-free media in Europe shortly after the introduction of avoparcin in European animal husbandry (7) or in more recently isolated North American strains (15). The VRE strains from animals described to date (1, 2, 12) have been isolated selectively on vancomycin-containing liquid or solid medium. The vancomycin selective enrichment procedures used in those studies and in the present work detect only the VanA phenotype. The vancomycin concentration of 20  $\mu\text{g/ml}$  of the enrichment broths is too high to allow for the growth of strains with the low-level glycopeptide resistance phenotypes VanB and VanC. The only *vanC*-positive strain, an *E. gallinarum* isolate recovered from an enrichment culture of the intestinal contents of a pheasant, was probably detected because it was additionally *vanA* positive. A similar strain has been described recently from a human patient taking vancomycin orally (5). As in humans, most VRE isolates identified here were *E. faecium*. Vancomycin-resistant *E. durans* strains have been described in two renal transplant patients (9), but apparently, such strains are rare in animals and humans.

Sample selection in the present study was determined by our primary aim, which was to study samples from as many different animal origins as possible. For this reason only one sample per farm or per owner was examined. The selection of the material may be biased because many samples originated from farms experiencing disease or increased mortality. However,

most diseases diagnosed in the necropsied animals included in the study were not due to bacteria and virtually none were due to enterococci. For some of the animal species less frequently kept as farm or pet animals, the number of samples was too low to allow us to draw conclusions regarding the prevalence of glycopeptide resistance. Nevertheless, it can be assumed that the material examined was fairly representative of the animal exposures that humans may have.

We concluded that *vanA*-mediated glycopeptide resistance is widespread in enterococci from animals, at least in the species *E. faecium*. Our results confirm and expand those of Bates and colleagues (2), who found vancomycin-resistant *E. faecium* strains in 15 of 36 pigs sampled at an experimental field station and in a duck, a chicken, a turkey, a dog, a pony, and 2 pigs on a single small farm.

Our purpose to examine material from diverse origins made it difficult to obtain dependable information on the often complex and variable antibiotic use in the animals and on the farms sampled. Although pet animals do not receive antibiotics in their food, at least in some of them acquired *vanA*-based resistance occurs. The possibility that these animals may have acquired these strains from meat products in their feeds originating from animals fed avoparcin or from fecally contaminated foods cannot be excluded. Whether feeding glycopeptide antibiotics such as avoparcin results in an increased incidence of acquired glycopeptide resistance, as has been suggested previously (1, 11), should be confirmed by carefully controlled studies. Answers to this question and investigations on the possible epidemiological relationships between animal and human glycopeptide-resistant enterococci, and notably *E. faecium*, are of utmost importance.

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