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Glycopeptide Susceptibility among Danish *Enterococcus faecium* and *Enterococcus faecalis* Isolates of Animal and Human Origin and PCR Identification of Genes within the VanA Cluster

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The MICs of vancomycin and avoparcin were determined for isolates of *Enterococcus faecium* and isolates of *Enterococcus faecalis* recovered from the feces of humans and animals in Denmark. Two hundred twenty-one of 376 (59%) isolates of *E. faecium* and 2 of 133 (1.5%) isolates of *E. faecalis* were resistant to vancomycin (MICs, 128 to ≥256 μg/ml), and all vancomycin-resistant isolates were resistant to avoparcin (MICs, 64 to ≥256 μg/ml). All vancomycin-resistant isolates examined carried the *vanA*, *vanX*, and *vanR* genes, suggesting that a gene cluster similar to that of the transposon Tn*1546* was responsible for the resistance.

Enterococcus faecalis and *Enterococcus faecium* isolates have emerged as important human pathogens that are especially responsible for nosocomial infections (14, 17), and with the emergence of vancomycin resistance, enterococci that are resistant to all currently approved antimicrobial agents may occur (5, 9). Recently, vancomycin-resistant enterococci have been isolated from the feces of poultry and pig herds (1, 3, 11), making dissemination via contaminated food products possible. These vancomycin-resistant *E. faecium* isolates from animals had decreased susceptibilities to avoparcin, a glycopeptide antibiotic widely used as a growth promoter in Western Europe and Australasia (5a). Avoparcin is a fermentation product from a strain of *Streptomyces candidus* and is closely related to vancomycin (12). Only limited information regarding the susceptibilities of *E. faecium* isolates to avoparcin is available, and no breakpoints for resistant versus susceptible isolates have ever been established. Redin and Dornbush (15) studied the in vitro activities of vancomycin and avoparcin and found MICs between 3 and 12 μg/ml for 16 group D streptococcal isolates. Dutta and Devriese (8) studied 15 isolates of *E. faecium* and 8 isolates of *E. faecalis* and found avoparcin MICs of from 0.5 to 2 μg/ml. In more recent studies, Klare et al. (10, 11) examined vancomycin-resistant isolates of *E. faecium* from pigs, poultry, the environment, foodstuffs, and humans for their susceptibilities to vancomycin and avoparcin. Those investigators found avoparcin MICs of from 8 to 512 μg/ml. In Denmark avoparcin has been used for growth promotion in pigs and poultry for almost 20 years and accounted for 21% of all growth-promoting antibiotics used in Denmark in 1994 (6). In a recent Danish study of 29 *E. faecium* isolates from poultry, avoparcin MICs ranged from 0.5 to 8 μg/ml for 19 vancomycin-susceptible isolates and from 128 to ≥256 μg/ml for 10 vancomycin-resistant isolates (1).

This paper describes the susceptibilities to vancomycin and avoparcin of a collection of *E. faecium* and *E. faecalis* isolates from the feces of animals and humans in Denmark, as determined by measuring the MICs for the isolates.

Isolates. From March to June 1995 fecal samples from humans and production animals in Denmark were examined for the presence of *E. faecium* and *E. faecalis* isolates, and the isolates were tested for their resistance to vancomycin and avoparcin. A total of 119 and 57 isolates of *E. faecium* and *E. faecalis*, respectively, from the feces of poultry from 51 chicken flocks, 210 and 20 isolates of *E. faecium* and *E. faecalis*, respectively, from the feces of pigs from 49 pig herds, and 24 and 35 isolates of *E. faecium* and *E. faecalis*, respectively, from feces of calves from 32 dairy herds were recovered. Furthermore, 23 and 21 *E. faecium* and *E. faecalis* isolates, respectively, from the feces of hospitalized humans with diarrhea were included in the study. Only one isolate per animal or human was included. All isolates were identified to the species level as described by Devriese et al. (7).

Susceptibility. The MICs of vancomycin and avoparcin (the antimicrobial agents were obtained from Eli Lilly, Indianapolis, Ind., and Cyanamide, Hampshire, England, respectively) were determined on Mueller-Hinton II agar (Mueller-Hinton II; Becton Dickinson Microbiology System, Cockeyville, Md.) according to the description provided by Sahm and Washington (16). *E. faecium* BM4147 (13), *E. faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *S. aureus* ATCC 25923 were used as quality control strains.

The MICs of vancomycin and avoparcin for 376 *E. faecium* and 133 *E. faecalis* isolates are provided in Table 1. The MICs for vancomycin-resistant isolates (MICs, ≥16 μg/ml) ranged from 128 to ≥256 μg/ml for vancomycin and from 64 to ≥256 μg/ml for avoparcin. The MICs for vancomycin-susceptible isolates ranged from 0.5 to 8 μg/ml for vancomycin and from 0.25 to 8 μg/ml for avoparcin. Isolates were either coresistant to both avoparcin and vancomycin or were susceptible to both antibiotics. A total of 221 isolates of *E. faecium* and 2 isolates of *E. faecalis* were found to be resistant to vancomycin and avoparcin. Vancomycin-resistant *E. faecium* isolates were found in 36 poultry flocks and 10 pig herds and among 2 of the human isolates, whereas no vancomycin-resistant *E. faecium* isolates were found among the isolates from calves. Vancomycin-resis-

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TABLE 1. Vancomycin and avoparcin MICs for 376 *E. faecium* and 133 *E. faecalis* isolates from human and animal sources in Denmark

Bacterial species	Antibiotic	No. of isolates for which MIC (μg/ml) was as follows[a]:									
		0.25	0.5	1	2	4	8	64	128	256	>256
E. faecium	Vancomycin		55	43[b,c]	23	32[d]	2		2	6	213[e]
	Avoparcin	1	1	30	47	74[c,d]	2[b]	4	108	29	80[e]
E. faecalis	Vancomycin			69	13	49					2
	Avoparcin			1	7	123					2

[a] For none of the isolates were MICs ≤0.125, 16, or 32 μg/ml.
[b] MIC for *S. aureus* ATCC 29213.
[c] MIC for *S. aureus* ATCC 25923.
[d] MIC for *E. faecalis* ATCC 29212.
[e] MIC for *E. faecium* BM4147.

tant *E. faecalis* isolates were detected only among isolates from two poultry flocks.

Detection of *vanA*. A maximum of two vancomycin-resistant isolates from each flock or herd were examined by PCR. Furthermore, 25 susceptible isolates from 25 flocks were included. A total of 85 vancomycin-resistant and 25 susceptible-isolates of *E. faecium* and 2 vancomycin-resistant *E. faecalis* isolates were tested for the presence of *vanA* by PCR. The primers were chosen from the published sequence of the *vanA* gene cluster (6). The primers for amplifying *vanA* were 5′-AATGT GCGAAAAACCTTGC-3′ and 5′-AACAACTAACGCGGC ACT-3′. The samples were amplified for 30 cycles, with each cycle consisting of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C, and standard buffer (Perkin-Elmer) was used. The PCR products were analyzed by agarose gel electrophoresis. *E. faecium* BM4147 was used as a positive control, and *E. faecalis* ATCC 29212 was used as a negative control. The identity of the amplification product of the *vanA* PCR was verified by sequencing the DNA of 10 isolates (18). All vancomycin-resistant isolates but none of the susceptible isolates gave positive reactions in the PCR. The DNA sequences of the PCR products of the 10 isolates examined were identical for all isolates and were similar to the sequence of the PCR product from *E. faecium* BM4147.

Detection of *vanR* and *vanX*. Twenty-five vancomycin-resistant *E. faecium* isolates were also examined for the presence of *vanR* and *vanX*. The primers for amplifying the *vanR* gene were 5′-AAATAAGGGACAAGCACACC-3′ and 5′-CCCAT ATCTCATGAAATAGC-3′, and the primers for amplifying the *vanX* gene were 5′-ACTTGGGATAATTTCACCGG-3′ and 5′-TGCGATTTTGCGCTTCATTG-3′. PCRs were performed as described above for *vanA*, with the modification that annealing was performed at 55°C. All isolates examined were positive by the PCR.

In the present study we found that isolates of *E. faecium* and *E. faecalis* could be divided into two distinct and corresponding groups by determination of the vancomycin and avoparcin MICs for the isolates. The presence of the *vanA*, *vanR*, and *vanX* genes in all resistant isolates examined suggests that a genetic structure similar to that described previously for transposon Tn*1546* (6) was responsible for the resistance.

The presence of VanA-mediated vancomycin resistance in *E. faecium* isolates from 36 poultry flocks and 10 pig herds suggests that this resistance is widespread among the isolates from poultry and pigs in Denmark. Vancomycin-resistant *E. faecium* isolates were not observed among the isolates from calves, and this is in agreement with the fact that even though avoparcin is approved for use in calves, it has never been used in calves in Denmark.

The concentration of avoparcin used in poultry feed in Denmark is 15 μg/g of body weight, whereas the concentrations used for pigs are 20 and 40 μg/g for weaners and finishers, respectively (4). Avoparcin is left virtually unabsorbed by the digestive system in chickens and pigs, and most of the active antibiotic passes through the digestive system without being metabolized (4). The concentration of avoparcin in the intestinal tracts of animals fed avoparcin as a feed additive probably exceeds the MIC of avoparcin for the group of vancomycin-susceptible isolates. The selective pressure caused by the use of avoparcin as a feed additive in favor of vancomycin-resistant *E. faecium* or *E. faecalis* isolates must consequently be strong, and the most likely explanation for the widespread presence of vancomycin-resistant enterococci among animals in Denmark is the use of avoparcin as a growth promoter. On the basis of the present and other studies, the use of avoparcin as a growth promoter for animals was banned by the Danish Ministry of Agriculture in 1995.

In conclusion, the present study showed that *E. faecium* isolates coresistant to vancomycin and avoparcin are commonly found in the feces of pigs and poultry in Denmark. The presence of the *vanA*, *vanX*, and *vanR* genes in all of the vancomycin-resistant isolates examined suggests that a gene cluster similar to that found on the transposon Tn*1546* is responsible for this resistance.

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