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Phenotypic Distinction in *Enterococcus faecium* and *Enterococcus faecalis* Strains between Susceptibility and Resistance to Growth-Enhancing Antibiotics

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Susceptibility of *Enterococcus faecium* and *Enterococcus faecalis* strains from animals and foods to growth-promoting antibiotics used in animal feed was tested by the agar dilution technique. Acquired resistance to bacitracin, narasin, tylosin, and virginiamycin was seen for both species, and for *E. faecium*, resistance to avilamycin and avoparcin was also seen. Drawing the distinction between susceptibility and resistance based on frequency distributions of MICs was easy with avoparcin, avilamycin, and tylosin but difficult with virginiamycin and to some extent also with bacitracin and narasin.

Antibiotic resistance in gram-positive cocci, especially enterococci, has received much attention in recent years. Glycopeptide (vancomycin)-resistant enterococci are nowadays a major problem in nocosomial infections in humans. The emergence and spread of this resistance have been attributed in Europe to the use of avoparcin (3), a glycopeptide antibiotic used until recently as a growth promoter in animal nutrition in the countries of the European Community but not in North America. Despite the widespread application of certain antibiotics mixed in the feed for growth enhancement of farm animals, little information on the in vitro susceptibility of their intestinal flora to these antibiotics is available. In the present study, we have investigated the distinction between susceptibility and resistance of *Enterococcus faecium* and *Enterococcus faecalis* to growth-enhancing antibiotics.

A total of 199 strains of *E. faecium* (47 from pet animals, 66 from farm animals, and 86 from foods) and 154 strains of *E. faecalis* (53 from pet animals, 62 from farm animals, and 39 from foods) were isolated in Belgium in 1996, 1997, and 1998. Each strain was representative of a single origin: a single farm, owner, or food batch. Samples were inoculated on Columbia blood agar with colistin and nalidixic acid (Gibco, Paisley, United Kingdom) supplemented with 5% ovine blood, Slanetz and Bartley agar (Oxoid, Basingstoke, United Kingdom), or kanamycin esculin azide agar (Oxoid). Enterococcus-like colonies were purified and identified as described earlier (5, 6, 8).

The following laboratory standard antibiotic preparations were tested: avoparcin (American Cyanamid, Princeton, N.J.), virginiamycin (Pfizer, Rixensart, Belgium), bacitracin (67,000 IU/g; Sigma, St. Louis, Mo.), tylosin (Sigma), avilamycin (Eli Lilly, Indianapolis, Ind.), and narasin (Eli Lilly). MIC tests were carried out as described previously (4) on unsupplemented Mueller-Hinton II medium (Becton Dickinson, Cockeysville, Md.) incubated aerobically. Three control strains, *E. faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and the *E. faecium* type strain LMG 11423^T, were included in the tests.

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Investigations on the antimicrobial activity of growth-enhancing antibiotics are hampered by the fact that guidelines for carrying out and interpreting the in vitro tests are not available (7). Susceptibility breakpoints have not been established. To aid interpretation, a table with MIC results obtained with internationally utilized control strains and the *E. faecium* type strain under the test conditions applied in this study has been added (Table 1). It should be noted that the tests were carried out in only one laboratory and that the National Committee for Clinical Laboratory Standards requires a study involving five to six different laboratories for the purposes of defining quality control ranges.

An even greater difficulty in the study of the antibacterial activity of agents used for growth promotion concerns the interpretation of MIC results in terms of sensitivity and resistance. MIC-blood level relationships cannot be used to guide interpretations as is customary with clinically used antibiotics. The inhibitory concentrations can be related to the intestinal concentrations of the antibacterials. These are largely unknown, but they are probably principally determined by the feed concentrations used, at least in the case of the unabsorbed antibiotics (7). When a bimodal frequency distribution of susceptibility levels is present among strains of a given species, a biological or microbiological criterion can be applied. The group with the lower MICs can be classified as susceptible, and

TABLE 1. MICs of growth-enhancing antibiotics for *S. aureus*, *E. faecalis*, and *E. faecium* control strains (medians and ranges as recorded in 12 repetitive tests)

Antibiotic	Result for organism[a]:					
	S. aureus (ATCC 29213)		*E. faecalis* (ATCC 29212)		*E. faecium* (LMG 14423^T)	
	Range	Median	Range	Median	Range	Median
Avilamycin	0.5–2	1	0.25–0.5	0.25	0.25	0.25
Avoparcin	4	4	2–4	4	2	2
Bacitracin	2	2	2	2	≤0.12–1	0.5
Tylosin	1–2	2	1–2	2	0.5	0.5
Virginiamycin	2–4	2	2–4	2	0.25–0.5	0.25
Narasin	0.25	0.25	0.25	0.25	≤0.12	≤0.12

[a] Results are expressed in micrograms per milliliter, except for those with bacitracin, which are in international units per milliliter.

TABLE 2. MICs of growth-enhancing antibiotics for *E. faecium* and *E. faecalis* strains from animals and foods

Antibiotic	Sp.	No. of strains with MIC[a]:									
		≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Avilamycin	*E. faecium*	13	50	105	27	2					2
	E. faecalis	1	22	90	40	1					
Avoparcin	*E. faecium*			7	115	70	4				2
	E. faecalis				8	111	31	4			
Bacitracin	*E. faecium*	15	24	14	21	44	49	2	7	10	14
	E. faecalis			17	11	43	59				24
Tylosin	*E. faecium*		1	10	51	43	3		1	22	68
	E. faecalis					37	21	12		1	83
Virginiamycin	*E. faecium*		27	36	50	44	26	7	9	7	3
	E. faecalis			2	6	58	54	25	7	1	1
Narasin	*E. faecium*	25	132	7	4	22	8	1			
	E. faecalis	12	133	7	1		2				

[a] Micrograms per milliliter, except that MICs of bacitracin are expressed in international units per milliliter.

the remaining strains can be considered as having acquired resistance, but the in vivo relevance of these purely microbiological distinctions is not clear.

Frequency distributions of MIC test results obtained with the *E. faecium* and *E. faecalis* strains tested are shown in Table 2. Two *E. faecium* strains were distinctly less susceptible to avilamycin than were the other strains examined, while avilamycin MICs for *E. faecalis* strains were monomodally distributed. A similar apparently acquired avilamycin resistance phenotype, the mechanism of which is unknown, has been described recently by Aarestrup (1). A large majority of strains were susceptible to avoparcin, but two strains with high avoparcin MICs had acquired *vanA*-mediated resistance, as evidenced by PCR typing of glycopeptide resistance genes (8).

The normal bacitracin sensitivity levels of *E. faecalis* and *E. faecium* were found to have a wide range: the MICs for possibly sensitive *E. faecalis* strains range from 0.5 to 4 IU/ml, and those for *E. faecium* range from equal to or less than 0.12 to 4 IU/ml. This extended range caused interpretative difficulties. We propose to consider 8 IU/ml as the critical level for both species.

The interpretation of the MIC frequency distributions of tylosin poses no difficulties. This situation is very different from that with the related streptogramin antibiotic virginiamycin. The extended virginiamycin MIC ranges are most probably caused by the individual or simultaneous occurrence of different resistance mechanisms affecting either virginiamycin S or virginiamycin M, or both components of this antibiotic mixture (2). Determining MICs of the single components or investigating resistance genes offers better possibilities for determining resistance.

The MICs for the narasin-resistant strains were bimodally distributed, which indicates acquired resistance. However, the MICs for resistant strains differed by only 2 to 5 twofold dilution steps from the sensitivity levels of the other field strains (Table 2) as well as those of the collection strains (Table 1). Hence, the term "decreased susceptibility" is perhaps more appropriate for this phenotype, which was seen more frequently for *E. faecium* than for *E. faecalis*. A critical level of 1 μg/ml can be used with this antibiotic to distinguish enterococci with acquired narasin resistance from susceptible strains.

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