Prevalence and Antimicrobial Resistance of *Enterococcus* Species Isolated from Retail Meats

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From March 2001 to June 2002, a total of 981 samples of retail raw meats (chicken, turkey, pork, and beef) were randomly obtained from 263 grocery stores in Iowa and cultured for the presence of *Enterococcus* spp. A total of 1,357 enterococcal isolates were recovered from the samples, with contamination rates ranging from 97% of pork samples to 100% of ground beef samples. *Enterococcus faecium* was the predominant species recovered (61%), followed by *E. faecalis* (29%), and *E. hirae* (5.7%). *E. faecium* was the predominant species recovered from ground turkey (60%), ground beef (65%), and chicken breast (79%), while *E. faecalis* was the predominant species recovered from pork chops (54%). The incidence of resistance to many production and therapeutic antimicrobials differed among enterococci recovered from retail meat samples. Resistance to quinupristin-dalfopristin, a human analogue of the production drug virginiamycin, was observed in 54, 27, 9, and 18% of *E. faecium* isolates from turkey, chicken, pork, and beef samples, respectively. No resistance to linezolid or vancomycin was observed, but high-level gentamicin resistance was observed in 4% of enterococci, the majority of which were recovered from poultry retail meats. Results indicate that *Enterococcus* spp. commonly contaminate retail meat sam that dissimilarities in antimicrobial agents in each food animal production class.

Protection of the food supply includes considerations of the microbiological quality and safety of commodities available for public consumption. While these concerns often address specific pathogenic microorganisms that present an immediate risk to public health, there is growing interest in commensal components of the flora associated with food-producing animals that may also impact consumers. Species of the genus *Enterococcus* comprise a large proportion of the autochthonous microflora associated with the gastrointestinal tracts of animals and are frequently responsible for significant morbidity and mortality in predisposed humans (27).

Enterococci are common components of the microfloral community of mammals, birds, insects, and reptiles and are commonly found in soil, on plants, and in water. These organisms are particularly challenging to eliminate because of their ability to adapt to environmental stresses. Thus, it is not surprising that antimicrobial-resistant variants of enterococci have been recovered from meats, dairy products, and ready-to-eat foods and have even been found within probiotic formulations (29). In the clinical environment, enterococci can persist for long periods of time on surfaces and can readily be transferred among the patient population, many of whom may be prone to colonization (46).

Enterococci, particularly *Enterococcus faecalis* and *E. faecium*, present serious challenges to the control of antimicrobial resistance as they are the third leading cause of nosocomial infections in intensive care units in the United States (18). Additionally, infections caused by other Enterococcus species (E. durans, E. avium, E. raffinosus, E. gallinarum, and E. casseliflavus) occasionally occur and warrant attention (44). Perhaps more importantly, enterococci are adept in acquiring and transferring elements that confer resistance to antimicrobials. In addition, they are known to be intrinsically resistant to several antibiotics. As a result, therapeutic options for treatment of enterococcal infections are increasingly limited (44). In 1980 the reported development of and subsequent increase in resistance to the glycopeptide vancomycin among clinical isolates of Enterococcus spp. was followed by a flurry of research into new antimicrobials for alternative therapy. Ironically, the 1999 Food and Drug Administration approval of the streptogramin quinupristin-dalfopristin (Q-D; Synercid) to treat vancomycin-resistant E. faecium infections came after more than 20 years of widespread use of the streptogramin analogue virginiamycin in animal production. This has revived concerns that use of antimicrobials in food animal production might compromise the efficacy of related drugs in human clinical medicine through selection of resistant populations and their subsequent transfer through the food supply (30).

Enterococci of food-borne origin have not been conclusively identified as direct causes of clinical infections; however, the consumption of meat carrying antibiotic-resistant bacterial populations is a possible route of transfer and could result in either colonization or transfer of resistance determinants to host-adapted strains. Data on the prevalence of antimicrobialresistant enterococci from retail food are unfortunately sparse in the United States and are urgently needed for scientific assessments of the relative risks of using antimicrobials in

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animal husbandry. The data reported here are the results of a pilot surveillance project undertaken in Iowa to determine the prevalence and antimicrobial resistance profiles of enterococci in retail meats.

MATERIALS AND METHODS

Sample collection. Between March 2001 and June 2002, 981 packages of retail turkey, chicken, pork, and beef were purchased from 263 separate grocery stores around Iowa. Turkey and beef samples were predominantly ground products, while samples of pork and chicken were predominantly whole cuts. Grocery locations in Iowa (supermarkets or superstores) were drawn from two databases, the Chain Stores Grocery Guide (Chain Store Guide, Tampa, Fla.) and the Single Unit Grocery Guide. These guide databases were filtered by sales volume to eliminate most of the nongrocery convenience-type stores. This list was inspected, and the obvious health food and convenience stores were eliminated. Field personnel sampled one package each of turkey, chicken, pork, and beef from six different supermarket stores on a weekly basis. Retail meat samples were sealed in a plastic bag, labeled with a unique identifying number, and placed into a cooler with ice packs. Field personnel transported the food specimens to Food and Drug Administration-Center for Veterinary Medicine laboratories in Laurel, Md., within 48 h of collection.

Sample processing and isolation of enterococci. Two hundred twenty-five milliliters of Enterococcosel enrichment broth (BBL Microbiology Systems, Cockeysville, Md.) was added to 25 g of aseptically weighed ground sample in a stomacher bag. Bags were stomached with a Stomacher 400 circulator (Seward, Inc., London, England) at 230 rpm for 2 min. Whole cuts were added to a sterile Whirl-Pak bag (Nasco, Fort Atkinson, Wis.), and at least 225 ml of Enterococcosel broth was added to cover the meat sample. Bags were placed on an Innova 2100 platform shaker (New Brunswick Scientific, Edison, N.J.) and shaken at 200 rpm for 15 min, followed by the aseptic removal of the whole cut. Enrichment broths were then closed and incubated in a water bath at 45°C and evaluated at 24 and 48 h for blackening of the culture broth. When blackening was observed, a 10-µl loop was used to streak the surface of an Enterococcosel agar plate, which was incubated at 35°C for 24 \pm 2 h. If no growth or no blackening was observed in the enrichment broth after 48 h of incubation, the culture was deemed negative and discarded. From each Enterococcosel agar plate, up to three colonies of distinctive morphology were streaked for isolation onto blood agar plates.

Identification of enterococci. Presumptive enterococci were identified on the basis of esculin hydrolysis, Gram stain, catalase reaction, and pyrrolidonyl arylamidase test results (BBL). Hemolytic reaction and pigmentation were also recorded. Use of the *Enterococcus* AccuProbe culture identification kit (Gen-Probe, Inc., San Diego, Calif.) was reserved for isolates that were ambiguously identified. The VITEK (bioMérieux, Inc., Hazelwood, Mo.) microbial identification system was routinely used to distinguish *Enterococcus* species. Supplementary testing included arabinose and sucrose utilization (Sigma-Aldrich, St. Louis, Mo.), as well as assays for motility and methyl- α -D-glucopyranosidase production (17). Isolates were frozen at -70° C in brucella broth with 20% glycerol.

Antimicrobial susceptibility testing of enterococci. Antibiograms for each of the enterococcal isolates were determined with the Sensititre antimicrobial susceptibility testing system for 17 antimicrobials (Trek Diagnostic Systems, Inc., Westlake, Ohio). The antimicrobials and tested ranges were as follows: bacitracin, 8 to 128 IU/ml); chloramphenicol, 2 to 32 µg/ml; ciprofloxacin, 0.12 to 4 µg/ml; erythromycin and linezolid, 0.5 to 8 µg/ml; bambermycin (Flavomycin), lincomycin, Q-D, and salinomycin, 1 to 32 µg/ml; nitrofurantoin, 2 to 128 µg/ml; penicillin, 0.5 to 16 µg/ml; tetracycline, 4 to 32 µg/ml; tylosin, 0.25 to 32 µg/ml; vancomycin, 0.5 to 32 µg/ml; gentamicin and kanamycin, 128 to 1,024 µg/ml; streptomycin, 512 to 2,048 µg/ml. Microtiter plates containing the tested antimicrobials with a final inoculum concentration of approximately 5×10^5 CFU/ml were incubated at 37°C for 24 \pm 1 h in ambient air. E. faecalis strains ATCC 29212 and ATCC 51299 were used as quality control organisms. The plates were removed and read manually for growth to score the MIC determinations using the following NCCLS breakpoints: chloramphenicol and vancomycin, ≥32 µg/ ml; erythromycin and linezolid, $\geq 8 \,\mu g/ml$; penicillin and tetracycline, $\geq 16 \,\mu g/ml$; Q-D and ciprofloxacin, $\ge 4 \ \mu g/ml$; nitrofurantoin, $\ge 128 \ \mu g/ml$; gentamicin, >500µg/ml; streptomycin, >1,000 µg/ml (45). Non-NCCLS resistance breakpoints for bacitracin (>64 IU/ml), tylosin (>8 µg/ml), bambermycin (>8 µg/ml), and salinomycin (>8 µg/ml) have been used elsewhere (3, 4, 45), while no breakpoint for lincomycin has been established. A breakpoint of >500 µg/ml was used for kanamycin. Enterococcal antibiograms recovered from different isolates from

TABLE 1. Prevalence of *Enterococcus* spp. among retail meat products from Iowa

Meat class	No. sampled	No. positive	% Positive
Turkey	227	226	99.6
Chicken	237	236	99.6
Pork	255	247	96.9
Beef	262	262	100.0
All meats	981	971	99.0

the same retail meat sample that differed by less than 2 dilutions for one or more antimicrobial MICs were considered duplicates, and only a single isolate was included for further analysis. Chi-square analysis was performed using commercial statistical analysis software (SAS Institute, Cary, N.C.) to determine significant differences in resistance rates among meat types as well as between populations *E. faecium* and *E. faecalis*.

RESULTS

Isolation and identification of enterococcal species. Enterococci were observed to be ubiquitous among retail meat products collected from Iowa, with the recovery of enterococci from 99% of 981 samples cultured (Table 1). Only 13 isolates were not identified to species. Resistance profiles were established for all 1,511 isolates except for 1 that did not grow in Mueller-Hinton broth. The collection was reduced to 1,357 unique isolates after the removal of isolates of the same species with nondistinct susceptibility patterns from the same meat sample. Among all meat classes, E. faecium (61%) was the most frequently encountered species, followed by E. faecalis (29%), E. hirae (5.7%), E. casseliflavus (2.1%), E. durans (1.2%), E. gallinarum (0.7%), and E. avium (0.1%), although differences in species prevalence varied by meat commodity (Table 2). Notably, E. faecium was the predominant species recovered from turkey, beef, and chicken meat, while E. faecalis accounted for the majority of isolates from pork. The predominance of E. faecium relative to E. faecalis was greatest among enterococci isolated from chicken (5:1), followed by beef (4:1) and turkey (2:1). E. casseliflavus and E. gallinarum were isolated more frequently from turkey than from other meat classes, while E. durans was recovered more frequently from pork and beef samples. Interestingly, E. hirae was more often recovered from beef than from the other meats analyzed.

Antimicrobial resistance of E. faecium and E. faecalis isolates. To assess the differences that might exist among Enterococcus spp. isolated from different meat products, the antimicrobial resistance profiles of the comparatively large populations of *E. faecium* (n = 825) and *E. faecalis* (n = 388) were examined (Table 3). The distributions of bacitracin MICs for E. faecium and E. faecalis were shifted to the upper range tested, with MICs for the majority of E. faecium isolates from turkey, chicken, and beef and E. faecalis isolates from turkey and chicken exceeding the upper limit (>128 µg/ml). Resistance to chloramphenicol was seen at a very low level (<1%) across the populations of E. faecium recovered, while a resistant subpopulation of E. faecalis was observed only among populations isolated from pork. Resistance to ciprofloxacin was observed at a higher frequency among E. faecium isolates than among E. faecalis isolates, with the greatest prevalence among E. faecium isolates recovered from turkey and chicken

Species		No. of <i>Enterococcus</i> sp., isolates (% of meat class isolates) in:								
	Turkey	Chicken	Pork	Beef	All meats					
E. avium	1 (0.3)	0	0	0	1 (0.1)					
E. casseliflavus	21 (5.9)	3 (1.0)	2 (0.7)	3 (0.8)	29 (2.1)					
E. durans	0	1(0.3)	7 (2.3)	8 (2.0)	16 (1.2)					
E. faecalis	110 (31)	51 (16)	161 (54)	66 (17)	388 (29)					
E. faecium	213 (60)	245 (79)	114 (38)	254 (65)	826 (61)					
E. gallinarum	6 (1.7)	0	1(0.3)	2(0.5)	9 (0.7)					
E. hirae	3 (0.8)	10 (3.2)	10 (3.4)	54 (14)	77 (5.7)					
Unidentified	3 (0.8)	1 (0.3)	3 (1.0)	4 (1.0)	11 (0.8)					
Total	357	311	298	391	1,357					

TABLE 2. Relative prevalence of Enterococcus spp. by retail meat class

(41 and 22%, respectively; P < 0.01). The ranges of MICs of ciprofloxacin for *E. faecium* isolates were more widely distributed than those for *E. faecalis*.

The distributions of MICs of the glycolipid antimicrobial bambermycin were relatively consistent among both species and did not appear to vary among retail meat commodities. MICs were consistently higher among *E. faecium* isolates (MIC at which 50% of isolates were inhibited [MIC₅₀] = >32 µg/ml) than among *E. faecalis* isolates (MIC₅₀ = 2 µg/ml), which may reflect species-specific intrinsic resistance to or tolerance of this antimicrobial (P < 0.01). This is contrasted with the MIC distributions for the ionophore salinomycin and the macrolides erythromycin and tylosin, which were elevated for both enterococcal species isolated from turkey and chicken meat (P < 0.01). Differences between species in the range of lincomycin MICs were similarly observed: a clustered distribution at the upper level of tested concentrations for *E. faecalis* isolates and a greater range among *E. faecium* isolates.

Resistance to nitrofurantoin was observed in one-half of all *E. faecium* isolates, while it was observed among only 5.5% of *E. faecalis* isolates from turkey. *E. faecium* isolates were also more often resistant to penicillin (P < 0.01), with the highest rates from turkey and chicken sources (P < 0.01). Tetracycline resistance was observed more frequently among *E. faecalis* isolates (P < 0.01), with the highest prevalence among both *E. faecium* and *E. faecalis* isolates from turkey, followed by those from pork, chicken, and beef.

Resistance to vancomycin or linezolid was not observed among *E. faecium* or *E. faecalis* isolates, but MICs for 48% of all *E. faecium* isolates were distributed 1 dilution away from clinical resistance to linezolid (MIC = 4 µg/ml). Over 94% of all *E. faecalis* isolates were resistant to the streptogramin Q-D, likely due to the purported intrinsic resistance of this species to this antimicrobial. Resistance to this streptogramin was highest among *E. faecium* isolated from turkey (54%), followed by chicken (27%), beef (18%), and pork (9%). It is notable that the distribution of MICs of Q-D for *E. faecium* of poultry origin revealed that the values were bimodally distributed and accounted for 76% of all resistant *E. faecium* isolates.

Antimicrobial resistance profiles of other *Enterococcus* spp. Among the less frequently recovered enterococcal species, decreased susceptibility to bambermycin was observed among all species, with some variability among *E. casseliflavus* and *E. gallinarum* populations (Table 4). Erythromycin resistance was observed in between 0 and 44% of the less frequently isolated enterococcal species. No striking differences among the MICs for these populations of bacitracin and salinomycin were observed although less variability in bacitracin MICs was observed among *E. casseliflavus* isolates. Resistance to tetracycline was frequent, with over 70% of all isolates displaying resistance, while resistance to nitrofurantoin was less common. No resistance to linezolid or vancomycin was observed; however, resistance to Q-D among 100, 41, 33, and 14% of *E. avium, E. casseliflavus, E. gallinarum,* and *E. hirae* isolates, respectively, was observed. No resistance to Q-D among *E. durans* isolates was observed. Similar to what was observed for *E. faecuum* and *E. faecalis*, 74% of these other species that were Q-D resistant were of poultry origin.

High-level aminoglycoside resistance among Enterococcus spp. Resistance to high-level aminoglycosides was prevalent across all species recovered (Table 5). Aside from the single isolate of *E. avium* that was resistant, the observed frequency of resistance to any of the three tested aminoglycosides was highest among isolates of *E. casseliflavus* (86%), followed by those of *E. faecium* (58%), *E. gallinarum* (56%), *E. durans* (38%), *E. faecalis* (17%), and *E. hirae* (12%). The patterns of susceptibility to high-level aminoglycosides were interesting in that resistance to kanamycin was the most prevalent, followed by resistance to streptomycin and resistance to gentamicin.

Upon closer examination of high-level aminoglycoside resistance among *E. faecalis* and *E. faecium* isolates, the resistance frequencies for both populations were highest for those that originated from poultry meat, with rates of 27, 33, 11, and 5% for *E. faecalis* and 74, 62, 41, and 47% for *E. faecium* isolates from turkey, chicken, pork, and beef, respectively (P < 0.01). Specifically, high-level gentamicin resistance was observed more frequently among isolates from poultry sources.

DISCUSSION

This work describes the distribution of enterococci among retail meat products from the Iowa and establishes a baseline for antimicrobial resistance among isolated *Enterococcus* spp. to antimicrobials of human and veterinary importance. Although we did not attempt to quantitate the enterococcal population within samples from Iowa in this study, the demonstration of near omnipresence of enterococci is likely reflective of a sizable population among the normal natural microflora of retail meat products. This is consistent with isolation rates of 82 to 86% from chickens reported from a previous study of a

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Antimicrobial and	Resistance		E. fac	ecium ($n = 825^c$)		E. faecalis $(n = 388^g)$			
meat class	breakpoint ^{a,b}	MIC ₅₀ ^a	MIC ₉₀ ^a	Range ^a	% Resistant	MIC ₅₀ ^a	MIC ₉₀ ^a	Range ^a	% Resistant
Bacitracin	>64								
Turkey		>128	>128	≤8–>128	96 ^{d,e}	>128	>128	32->128	$84^{d,e}$
Chicken		>128	>128	≤8–>128	98	>128	>128	64->128	90
Pork		128	>128	≤8–>128	72	128	>128	≤8–>128	68
Beef		>128	>128	≤8->128	88	128	>128	32->128	73
Bambermycin	>8								
Turkey		>32	>32	≤1->32	100^{e}	2	4	≤1–4	0^e
Chicken		>32	>32	16->32	100	2	4	≤1->32	2.0
Pork		>32	>32	2->32	99	2	4	≤1->32	1.9
Beef		>32	>32	>32	100	2	4	≤1-8	0
Chloramphenicol	>16								
Turkey		8	16	≤2->32	0.9	8	8	8-16	0
Chicken		8	8	4->32	0.4	8	8	8-16	0
Pork		8	8	8-32	0.9	8	8	8->32	3.1
Beef		8	8	8->32	0.4	8	8	8–16	0
Ciprofloxacin	≥ 4								
Turkey	-	2	>4	0.25->4	$41^{d,e}$	1	2	0.5–2	0^e
Chicken		2	4	0.25->4	22	1	2	0.5 - 2	0
Pork		1	2	0.25 -> 4	7.0	1	2	0.5–4	0.6
Beef		1	4	≤0.12->4	19	1	2	0.5–2	0
Erythromycin	≥ 8								
Turkey		8	> 8	$\leq 0.5 -> 8$	53^{d}	1	>8	$\leq 0.5 -> 8$	42^{d}
Chicken		2	>8	$\leq 0.5 -> 8$	20	1	>8	0.5 -> 8	33
Pork		2	4	$\leq 0.5 -> 8$	9.6	≤0.5	2	$\leq 0.5 -> 8$	8.1
Beef		2	4	≤0.5->8	8.7	1	2	≤0.5->8	4.5
Lincomycin	\mathbf{NA}^{f}								
Turkey		>32	>32	≤1->32	NA	>32	>32	16->32	NA
Chicken		>32	>32	≤1->32	NA	32	>32	≤1->32	NA
Pork		8	32	≤1->32	NA	32	>32	≤1->32	NA
Beef		16	32	≤1->32	NA	32	>32	4->32	NA
Linezolid	≥ 8								
Turkey		2	4	≤0.5–4	0	2	2	1–4	0
Chicken		2	4	≤0.5–4	0	2	2	2-4	0
Pork		2	4	≤0.5–4	0	2	2	≤0.5–4	0
Beef		4	4	≤0.5-4	0	2	2	2	0
Nitrofurantoin	≥128								
Turkey		64	128	8->128	50^e	16	32	8-128	5.5^{e}
Chicken		128	>128	16->128	55	16	16	8-64	0
Pork		64	128	16->128	41	16	16	8-64	0
Beef		128	128	32->128	51	16	16	8-64	0
Penicillin	>8								
Turkey		16	>16	≤0.5->16	54 ^{<i>d</i>,<i>e</i>}	4	4	2-8	0^e
Chicken		4	>16	≤0.5->16	23	4	4	2-4	0
Pork		2	8	≤0.5->16	4.4	4	4	2-16	0.6
Beef		4	8	≤0.5->16	2.8	4	4	2–8	0
Q-D	≥4								
Turkey		4	32	≤1-32	$54^{d,e}$	8	8	4-16	100^{e}
Chicken		2	16	≤1-32	27	8	8	≤1-16	96
Pork		2	2	≤1-8	8.8	8	8	≤1-16	95
Beef		2	4	≤1-16	18	8	8	≤1-16	97
Salinomycin	>8								
Turkey		2	8	≤1-8	0	≤1	4	≤1-8	0
Chicken		4	8	≤1-16	1.2	2	4	≤1-16	2.0
Pork		2	2	≤1-4	0	≤1	≤1	≤1-2	0
Beef		2	2	≤1-8	0	≤1	2	≤1–4	0

TABLE 3. Antimicrobial resistance profiles of E. faecium and E. faecalis isolates from retail meats

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Antimicrobial and meat class	Resistance breakpoint ^{<i>a,b</i>}	E. faecium $(n = 825^c)$			E. faecalis $(n = 388^g)$				
		MIC ₅₀ ^a	MIC ₉₀ ^a	Range ^a	% Resistant	MIC ₅₀ ^a	MIC ₉₀ ^a	Range ^a	% Resistant
Tetracycline	>8								
Turkey		>32	>32	≤4–>32	$87^{d,e}$	>32	>32	≤4–>32	94 ^{d,e}
Chicken		≤ 4	>32	≤4–>32	43	>32	>32	≤4–>32	67
Pork		32	>32	≤4–>32	60	>32	>32	≤4–>32	89
Beef		≤4	>32	≤4–>32	39	≤4	>32	≤4–>32	39
Tylosin	>8								
Turkey		8	>32	1->32	37	2	>32	1->32	42
Chicken		4	>32	1->32	16	2	>32	1->32	33
Pork		4	8	1->32	7.0	2	4	1->32	8.1
Beef		8	16	≤0.25->32	14	2	4	1->32	4.6

TABLE 3—Continued

^a Expressed in micrograms per milliliter except for bacitracin, for which the units are international units (IU) per milliliter.

^b Resistance breakpoints were those provided by NCCLS for chloramphenicol, ciprofloxacin, erythromycin, linezolid, nitrofurantoin, penicillin, Q-D, and tetracycline (47) and those suggested for the bambermycin and salinomycin (3) and tylosin and bacitracin (4).

^c 213, 244, 114, and 254 isolates from turkey, chicken, pork, and beef, respectively.

^d Denotes statistically significant differences among isolates from the different meat types in resistance to the indicated antimicrobial (P < 0.01).

^e Denotes statistically significant differences between E. faecium and E. faecalis isolates in resistance to the indicated antimicrobial (P < 0.01).

^fNA, not applicable (no established NCCLS breakpoint).

^g 110, 51, 161, and 66 isolates from turkey, chicken, pork, and beef, respectively.

wider geographical area (43). Indeed, studies of cooked poultry meat suggest that enterococci do not constitute the largest bacterial population on such products (9). While no study has previously determined the relative proportions of *Enterococcus* spp. from multiple meat types in the United States, *E. faecalis* has been observed more frequently among a limited number of frozen chicken samples from Michigan (53). The predominance of *E. faecalis* on retail pork products is consistent with studies of the enterococcal microflora of pork carcasses at U.S. processing facilities (40), although the influence of cultural methodology on the recovered population of enterococci is important (15).

Comparatively decreased susceptibility among *E. faecium* isolates, compared to *E. faecalis* isolates, to the glycolipid bambermycin has been previously ascribed to intrinsic resistance differences between the two species (16, 24, 25) although reduced tolerance among *E. faecium* isolates from unexposed environments suggests otherwise (4). The prevalence of chloramphenicol resistance has been reported more often among *E. faecalis* isolates than among *E. faecium* isolates from production environments (2, 58) and raw meat products abroad (28, 38, 49), while rates of resistance among *E. faecium* isolates to ciprofloxacin, erythromycin, nitrofurantoin, penicillin, and tetracycline are traditionally higher (26, 28, 47–49).

The observation of decreased susceptibility of E. faecium isolates, compared to E. faecalis isolates, to salinomycin seen in this study, especially those of poultry origin, is consistent with previous ionophore susceptibility results from production environments of Denmark (3) but differs from results for isolates of broiler origin from Japan (58) and Belgium (16). Similarly, the decreased relative susceptibility of E. faecium of poultry origin to bacitracin is most similar to the distributions of MICs for enterococci from of chicken and swine from Denmark, Finland, and Norway (4) but differs from those for enterococci from Belgium (16). The frequencies of resistance to high-level aminoglycosides among the more clinically relevant Enterococcus spp. from food animal production environments, especially among E. faecalis isolates, are often reported (22); however, the increased prevalence of gentamicin resistance among E. faecalis and E. faecium isolates from poultry meat seen in this study is inconsistent with the observations of enterococci from different production environments from Denmark and Belgium (2, 16). While data from comparable sources are few, these geographical differences likely reflect differences in antimicrobial use in food animal production practices.

Surveillance of enterococci from food sources for resistance to the oxazolidinone linezolid has not been reported previously. Resistance among isolates of *E. faecium* that are resis-

TABLE 4. MIC range and resistance profiles of *Enterococcus* spp. other than *E. faecalis* and *E. faecium* from retail meat for selected antimicrobials^a

Species (n)	MIC range ^{b} (% of isolates resistant) of:								
species (n)	BAC	BMB	ERY	LNZ	NIT	Q-D	SAL	TET	
<i>E. avium</i> (1) <i>E. casseliflavus</i> (29) <i>E. durans</i> (16) <i>E. gallinarum</i> (9) <i>E. hirae</i> (77)	>128 (100) 128->128 (100) ≤ 8 ->128 (69) ≤ 8 ->128 (78) ≤ 8 ->128 (22)	$ \begin{array}{c} >32 \ (100) \\ 2->32 \ (97) \\ 32->32 \ (100) \\ \leq 1->32 \ (67) \\ 32->32 \ (100) \end{array} $	$\begin{array}{c} 2 (0) \\ \leq 0.5 -> 8 (31) \\ \leq 0.5 -4 (0) \\ \leq 0.5 -> 8 (44) \\ \leq 0.5 -> 8 (17) \end{array}$	$\begin{array}{c} 2 (0) \\ 2-4 (0) \\ 2-4 (0) \\ 1-4 (0) \\ \leq 0.5-4 (0) \end{array}$	128 (100) 16->128 (55) 16->128 (38) 4-128 (22) 16->128 (10)	$\begin{array}{c} 8 (100) \\ \leq 1 - 32 (41) \\ \leq 1 - 2 (0) \\ \leq 1 - 32 (33) \\ \leq 1 - 16 (14) \end{array}$		$>32 (100) \\ \leq 4 ->32 (79) \\ \leq 4 ->32 (75) \\ \leq 4 ->32 (89) \\ \leq 4 ->32 (71)$	

^{*a*} BAC, bacitracin; ERY, erythromycin; BMB, bambermycin; LNZ, linezolid; NIT, nitrofurantoin; SAL, salinomycin; TET, tetracycline. Resistance breakpoints for *Enterococcus* spp. were those used by NCCLS (47) for erythromycin ($\geq 8 \mu g/ml$), linezolid ($\geq 8 \mu g/ml$), nitrofurantoin ($\geq 128 \mu g/ml$), Q-D ($\geq 4 \mu g/ml$), and tetracycline ($\geq 8 \mu g/ml$), with the exception of >8 $\mu g/ml$ for the bambermycin and salinomycin (3) and >64 IU/ml for bacitracin (4).

^b Expressed in international units per milliliter for bacitracin and in micrograms per milliliter for all other antimicrobials.

 TABLE 5. Frequency of high-level aminoglycoside resistance of Enterococcus spp. from retail meat

Species (n) and meat	No. (%) of isolates resistant to ^{a} :						
class	HLK	HLS	HLG				
E. avium (1)	1 (100)	0	0				
E. casseliflavus (29)	25 (86)	12 (41)	3 (10)				
E. durans (16)	6 (38)	0 ` ´	0 `				
E. faecalis (388)	$45(12)^{b,c}$	$44 (11)^{b,c}$	$26 (6.7)^c$				
Turkey	24 (53)	21 (48)	13 (50)				
Chicken	12 (27)	8 (18)	9 (35)				
Pork	8 (18)	12 (27)	4 (15)				
Beef	1 (2.2)	3 (6.8)	0				
<i>E. faecium</i> (825)	$414(50)^{b,c}$	$175(21)^{b,c}$	$26 (3.2)^c$				
Turkey	137 (33)	84 (48)	15 (58)				
Chicken	114 (28)	63 (36)	10 (38)				
Pork	47 (11)	18 (10)	0				
Beef	116 (28)	10 (5.7)	1 (3.8)				
E. gallinarum (9)	5 (56)	3 (33)	2 (22)				
E. hirae (77)	8 (10)	5 (6.5)	0				
Unidentified (11)	7 (64)	1 (9.1)	2 (18)				
Total	511 (38)	240 (18)	59 (4.4)				

^{*a*} Resistance breakpoints for *Enterococcus* spp. were $>500 \ \mu$ g/ml for high-level kanamycin (HLK), $>1,000 \ \mu$ g/ml for high-level streptomycin (HLS), and $>500 \ \mu$ g/ml for high-level gentamicin (HLG).

^{*b*} Denotes statistically significant differences among isolates from the different meat types in resistance to the indicated antimicrobial (P < 0.01).

^c Denotes statistically significant differences between *E. faecuum* and *E. faecalis* isolates in resistance to the indicated antimicrobial (P < 0.01).

tant to many antimicrobials has been observed (10; R. D. Gonzales, P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten, and J. P. Quinn, Letter, Lancet **357**:1179, 2001) and at least in one case without prior exposure (10). Additionally, isolates have been observed to develop resistance during the course of treatment (6, 34) and exhibit cross-resistance to other oxazolidinones (34). The increased MICs for *E. faecium* suggest that the development of clinical resistance among isolates of this species may not be a difficult adaptation following increased clinical usage of this antimicrobial in human clinical medicine.

Resistance to Q-D among food animal production environments in the United States is not surprising, given the use of the analogue virginiamycin since 1974 (32, 57). The higher frequency of Q-D resistance among E. faecium isolates from turkey than from chicken might be related to the different periods of time that the flocks are exposed to antimicrobials prior to slaughter (32). The resistance rate of 3% of E. faecium isolates from raw chicken samples reported from an earlier surveillance study in the United States using comparable nonselective enrichment methods (43) is much lower than the 26% observed among samples from this study. E. faecalis isolates have been shown to be intrinsically resistant to streptogramins (51); however, the recent observation of transferable resistance may lend some significance to the resistance seen among other species in this study (50). While the agricultural usage of antimicrobials that have analogues in human medicine is a matter of increasing public concern, resistance among E. faecium isolates from clinical environments has been shown to be higher than resistance among those from the community (23), which may or may not reflect similar selection in the clinical environment.

The absence of vancomycin-resistant enterococci (VRE) from domestic retail meats in this study is consistent with previous observations (21, 39, 53; Y. Ike, K. Tanimoto, Y. Ozawa, T. Nomura, S. Fujimoto, and H. Tomita, Letter, Lancet 353:1854, 1999) and reflects the absence of isolation of VRE from both processing (12) and food animal production environments (31, 35, 53, 57) in the United States. In contrast, VRE are frequently isolated from retail meat products (11, 36-38, 42, 49, 55, 56) from European countries as a result of selection of resistant populations by the use of the glycopeptide avoparcin in food animal production environments (1, 4, 5, 5)8, 16, 24, 52, 54). The persistence of VRE on farms that have discontinued the use of avoparcin for growth promotion illustrates the impact posed by antimicrobial usage in food animal production environments (7, 13, 14, 33, 41). It is clear that resistant enterococci recovered from raw meat products reflect this use of antimicrobials, but the extent to which these populations pose a risk to the consumer and the efficacy of therapeutic antimicrobials to treat disease is unknown. The recent observations of vancomycin resistance elements of enterococcal origin in U.S. clinical isolates of Staphylococcus aureus suggest that alternative therapies, such as linezolid and Q-D, should be more frequently employed (19, 20). As a result, resistant populations of enterococci that may have entered the human microflora through the consumption of contaminated retail meat products may be amplified as a result of the inevitable increase in selective pressure in the clinical environment.

Although existing evidence does not suggest that enterococci of food-borne origin be regarded as bacterial pathogens, they could serve as potential reservoirs of virulence and antimicrobial resistance genes for host-adapted strains. Our observations suggest that *Enterococcus* spp. commonly contaminate retail meat products and that differences observed in antimicrobial susceptibility phenotypes may reflect the extent of use of antimicrobials in specific food animal production environments. Therefore, effective control strategies aimed at reducing enterococcal contamination of retail meats may become more significant in the future, with increasing recognition of these bacteria as human opportunistic pathogens.

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