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Multiple-Antibiotic Resistance of *Enterococcus* spp. Isolated from Commercial Poultry Production Environments

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The potential impact of food animals in the production environment on the bacterial population as a result of antimicrobial drug use for growth enhancement continues to be a cause for concern. Enterococci from 82 farms within a poultry production region on the eastern seaboard were isolated to establish a baseline of susceptibility profiles for a number of antimicrobials used in production as well as clinical environments. Of the 541 isolates recovered, *Enterococcus faecalis* (53%) and *E. faecium* (31%) were the predominant species, while multiresistant antimicrobial phenotypes were observed among all species. The prevalence of resistance among isolates of *E. faecalis* was comparatively higher among lincosamide, macrolide, and tetracycline antimicrobials, while isolates of *E. faecium* were observed to be more frequently resistant to fluoroquinolones and penicillins. Notably, 63% of the *E. faecium* isolates were resistant to the streptogramin quinupristin-dalfopristin, while high-level gentamicin resistance was observed only among the *E. faecalis* population, of which 7% of the isolates were resistant. The primary observations are that enterococci can be frequently isolated from the poultry production environment and can be multiresistant to antimicrobials used in human medicine. The high frequency with which resistant enterococci are isolated from this environment suggests that these organisms might be useful as sentinels to monitor the development of resistance resulting from the usage of antimicrobial agents in animal production.

Our anthropocentric view of human pathogens has historically caused us to think of bacterial resistance to antimicrobials as a problem arising purely out of clinically related events. In fact, it is being increasingly recognized that antimicrobial resistance develops at a high frequency among bacteria in the food animal production environment. The conundrum is whether the prevalence of resistance in this environment contributes to the problem being observed in the clinical setting. *Enterococcus* spp., particularly *E. faecalis* and *E. faecium*, have presented serious challenges clinically, as they are the third leading cause of nosocomial infections in intensive care units in the United States and are becoming increasingly resistant to treatment with antimicrobials (8). Over 24% of nosocomial infections are complicated by the intrinsic resistance of this group of organisms to many antibiotics as well as acquired resistance to vancomycin (19).

Past surveillance has demonstrated a high prevalence of vancomycin-resistant enterococci (VRE) in the food animal production environments of the European Union (EU) as opposed to those of the United States, where no VRE have been reported from studies of farms in the United States (24, 39). These observations strongly implicate the agricultural use of the glycopeptide avoparcin in EU animal production in the development of resistance, which is thought be largely responsible for the increased prevalence of VRE in nonhospitalized human (community) populations of EU member nations compared with those from the United States (11). Similarly, the higher rate of occurrence of VRE among hospitalized patients in the United States have been ascribed to the extensive use of vancomycin in the hospital environment (28, 39).

Increased concern over selection for resistance through the use of analogues of human antimicrobials for growth promotion in animals has led the EU to ban the use of all antimicrobials as feed additives. The 1999 U.S. Food and Drug Administration approval of quinupristin-dalfopristin (Q-D or Synercid) for treatment of vancomycin-resistant *E. faecium* infections in humans has been met with similar concern due to the use of the analogue virginiamycin in agriculture in the United States for over 25 years. The demonstration of resistance in the food animal production environment (14, 18, 40), food products (17, 34), and the community (25) has raised concerns about the continued efficacy of this and other drugs in the clinical environment.

While the extent to which the selection and distribution of resistant human pathogens is related to the use of antimicrobials in agricultural is still hotly debated, few studies have actually detailed the multiresistant nature of enterococci from the food animal production environment to drugs used in production as well as human therapy. We therefore endeavored to characterize, in an unprecedented study, the species and related broad antimicrobial susceptibility profiles of a large number of *Enterococcus* spp. isolated from numerous poultry production operations located on the eastern seaboard of the United States.

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TABLE 1. Characterization of selected multiresistance phenotypes of *Enterococcus* spp. from the poultry production environment

Species or group	No. of isolates (% of total)[a]	% frequency distribution of resistance phenotype[b]																			
		L	LM	LP	LS	LT	LMP	LMS	LMT	LPS	LPT	LST	LMPS	LMPT	LMST	LPST	LMPST	M	MP	P	PT
E. avium	1 (0.3)								100												
E. casseliflavus	4 (1.2)					25					25					25	25				
E. durans	5 (1.5)								80								20				
E. faecalis	176 (53.2)				6.8			2.3	4.0			24			63						
E. faecium	104 (31.4)	2.9	1.0	2.9		1.0	8.7		3.8	1.0	11	14	1.0	1.0	4.8	32	11	1.0	1.9	1.0	1.0
E. gallinarum	20 (6.0)	5.0	25			30									15	15	10				
E. hirae	13 (3.9)	7.7	23		23				7.7		7.7	7.7			7.7		15				
Group II[c]	5 (1.5)				20							80									
Group III[c]	3 (0.9)				66							33									
Total	331 (100)	1.5	2.7	0.9	5.4	2.4	2.7	1.2	5.1	0.3	3.9	19	0.3	0.3	36	11	5.1	0.3	0.6	0.3	0.3

[a] Isolate numbers represent the data set subsequent to the removal of apparent nondistinct isolates recovered from a given sample.
[b] Resistance phenotype as defined by break points of ≥16 μg/ml for lincomycin (L), >4 μg/ml for erythromycin (M), >8 μg/ml for penicillin (P), ≥4 μg/ml for quinupristin-dalfopristin (S), and >8 μg/ml for tetracycline (T).
[c] As defined by Facklam and Collins (13).

MATERIALS AND METHODS

Sample collection. Samples consisted of either poultry litter or swabs of poultry transport containers. Surface poultry litter was collected from 55 roaster and broiler chicken houses located on the eastern seaboard of the United States, as described previously (16). Over a period of 11 weeks in the summer of 1998, two swabs from each of 103 poultry transport containers representing 27 farms were periodically collected at a regional processing facility. Fecal material from six surface sites of each of the poultry transport containers at the facility was swabbed with sterile gauze by using a 5-in.-diameter metal template, as described previously (32). Swabs were immersed in 50 ml of Cary Blair medium in sterile specimen cups, and litter samples were stored in sealed Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) and transported to the laboratory. Data on the identity and quantity of antimicrobials used among the production environments were not available.

Isolation and identification. Surface poultry litter was added at a 1:4 dilution to 40 ml of nalidixic acid-brain heart infusion-salt enrichment broth for incubation at 35°C with agitation in a Series 25 rotary shaker incubator (New Brunswick Scientific, Edison, N.J.). Similarly, swabs of the poultry containers were removed from the Cary-Blair medium, pooled in sets of three, and placed in 40 ml of nalidixic acid-brain heart infusion-salt broth and incubated as described above. Enterococci were cultured by transferring 100 μl of broth to colistin-nalidixic acid agar (Difco). From each colistin-nalidixic acid plate, up to three colonies of distinctive morphology were isolated. Isolates were presumptively characterized as enterococci based on Gram stain, catalase reaction, tolerance to 6.5% NaCl and growth at 45°C, the production of pyrrolidonyl arylamidase, and hydrolysis of esculin in the presence of bile. Confirmation to the genus level was accomplished by using an *Enterococcus* AccuProbe culture identification kit (GenProbe, Inc., San Diego, Calif.) according to the manufacturer's specifications.

Identification to species or group was done with a miniaturized identification system based on the biochemical tests recommended by Facklam and Collins (13), which included *R*-mannitol, *R*-sorbitol, L-sorbose, *R*-raffinose, and L-(+)-arabinose (Sigma-Aldrich, St. Louis, Mo.), performed with Costar 96-well cell culture plates (Corning, Inc., Corning, N.Y.), as well as assays for the presence of methyl-α-D-glucopyranosidase and arginine dihydrolase. Supplementary testing included ribose, sucrose, and inulin utilization as well as assays for motility. Control strains used in identification included American Type Culture Collection strains *E. faecalis* ATCC 51299, *E. avium* ATCC 35665, *E. pseudoavium* ATCC 49372, *E. raffinosus* ATCC 49427, *E. malodoratus* ATCC 43197, *E. faecium* ATCC 35667, *E. mundtii* ATCC 43186, *E. casseliflavus* ATCC 25788, *E. gallinarum* ATCC 49573, *E. durans* ATCC 49479, *E. hirae* ATCC 10541, *E. dispar* ATCC 51266, and *E. sulfureus* ATCC 49903. The VITEK (bioMérieux) microbial identification system was used to supplement identification. Isolates and control strains were frozen at −80°C in Trypticase soy broth supplemented with 20% glycerol.

Susceptibility testing. The MICs of 17 antimicrobials were determined for each of the isolates by using the Sensititre antimicrobial susceptibility testing system (Trek Diagnostic Systems, Inc., Westlake, Ohio). The antimicrobials and tested ranges included the following: bambermycin, 0.5 to 32 μg/ml; chloramphenicol, 2 to 64 μg/ml; ciprofloxacin, 0.06 to 4 μg/ml; clindamycin, 0.5 to 2 μg/ml; lincomycin, 1 to 32 μg/ml; erythromycin, 0.12 to 32 μg/ml; tylosin, 1 to 32 μg/ml; ampicillin, 0.25 to 16 μg/ml; penicillin, 0.25 to 128 μg/ml; bacitracin, 8 to 256 IU/ml; quinupristin-dalfopristin, 0.5 to 32 μg/ml; virginiamycin, 0.5 to 32 μg/ml; tetracycline, 0.25 to 32 μg/ml; vancomycin, 0.5 to 32 μg/ml; gentamicin and streptomycin, 128 to 2,048 μg/ml; and kanamycin, 64 to 2,048 μg/ml. Fifty microliters of a culture suspension in Mueller-Hinton broth containing approximately 5×10^5 CFU of each isolate/ml was inoculated into microtiter plates containing the test antimicrobials and incubated at 37°C for 18 h ± 1 h in ambient air. *E. faecalis* strains ATCC 29212 and ATCC 51299 were used as quality controls. The plates were removed and read manually for growth to score the MIC determinations by using the following NCCLS breakpoints: chloramphenicol and vancomycin, ≥32 μg/ml; erythromycin, ≥8 μg/ml; penicillin and tetracycline, ≥16 μg/ml; quinupristin-dalfopristin and ciprofloxacin, ≥4 μg/ml; gentamicin, >500 μg/ml; and streptomycin, >1,000 μg/ml (29). A breakpoint of >500 μg/ml was used for kanamycin. No NCCLS breakpoints have been established for bambermycin, lincomycin, tylosin, bacitracin, and virginiamycin. Strains of identical species from the same farm having common antibiograms, i.e., that differed by less than two dilutions for one or more MIC of a given antimicrobial, were considered to be duplicate isolates and only a single representative isolate was included for further analyses.

RESULTS

Characterization of enterococcal isolates. A total of 541 isolates of enterococci were recovered from the litter and crate swab samples from 82 farms. All isolates were identified to species level, and antimicrobial susceptibility profiles were established. This collection was reduced to 331 unique isolates after the removal of isolates of the same species from the same farm with essentially the same susceptibility patterns. There were no apparent differences in the species prevalence or their associated susceptibility profiles of enterococci isolated from litter and from poultry transport containers. *E. faecalis* was the predominant species (53.2%) identified followed by *E. faecium* (31.4%), *E. gallinarum* (6.0%), *E. hirae* (3.9%), *E. durans* (1.5%), *E. casseliflavus* (1.2%), and *E. avium* (0.3%). Eight isolates that were not clearly identifiable to the species level when biochemical means were used were placed into groups established by Facklam and Collins (13) based on the fermentation of mannitol and the activity of arginine dihydrolase.

Multiresistance phenotypes of *Enterococcus* spp. Multiple-drug resistance to antimicrobials used in the poultry production environment was prevalent among the isolates (Table 1). Reduced susceptibility to lincosamide antimicrobials was most often encountered, occurring in 98.5% of all species, followed by streptogramin (78.3%), tetracycline (68.0%), macrolide

TABLE 2. Frequency of high-level aminoglycoside resistance patterns of *Enterococcus* spp. from the poultry production environment

Species or group	No. of isolates with resistance phenotype(s) (% of species)[b]			
	HLS	HLK	HLS + HLK	HLK + HLG
E. casseliflavus	1 (25)	0	1 (25)	0
E. durans	1 (20)	1 (20)	0	0
E. faecalis	62 (35)	15 (8.5)	5 (2.8)	13 (7.4)
E. faecium	29 (28)	28 (27)	13 (13)	0
E. gallinarum	7 (35)	1 (5.0)	1 (5.0)	0
E. hirae	1 (7.7)	2 (15)	1 (7.7)	0
Group II[a]	1 (20)	0	0	0
Group III[a]	2 (67)	0	0	0
Total	104 (31)	47 (14)	21 (6.3)	13 (3.9)

[a] As defined by Facklam and Collins (13).
[b] Resistance breakpoints for *Enterococcus* spp. were >1,000 μg/ml for high-level streptomycin (HLS) resistance and >500 μg/ml for high-level kanamycin (HLK) and high-level gentamicin (HLG) resistance.

(54.3%), and penicillin (26.7%). No isolate was resistant to all five classes examined, but 52.7% were coresistant to four antimicrobials. Profiles of multiresistance of isolates to the selected antimicrobials were quite diverse with the lincosamide-macrolide-streptogramin-tetracycline (36%), lincosamide-streptogramin-tetracycline (19%), and lincosamide-penicillin-streptogramin-tetracycline (11%) phenotypes, most commonly observed on a percentage basis owing largely to the purported intrinsic resistance of the large number of *E. faecalis* isolates to streptogramin antimicrobials in the data set. The lincosamide-penicillin-streptogramin-tetracycline (23%), lincosamide-streptogramin-tetracycline (14%), and lincosamide-macrolide-penicillin-streptogramin-tetracycline (11%) resistance phenotypes were otherwise the most common multiresistance patterns observed among all isolates. Isolates of *E. faecium* demonstrated the largest diversity of multiresistance phenotypes ($n = 18$), followed by *E. hirae* ($n = 8$) and *E. gallinarum* ($n = 6$), compared to the five observed among the larger population of *E. faecalis* isolates. There were no observed isolates of vancomycin-resistant *E. faecium* or *E. faecalis*.

Resistance to high-level aminoglycosides was prevalent across all species except for a single isolate of *E. avium* (Table 2). The observed frequency of resistance was highest among isolates of *E. faecium* (68%), followed by group III *Enterococcus* spp. (67%), *E. faecalis* (53.7%), *E. casseliflavus* (50%), *E. durans* and *E. gallinarum* (40%), *E. hirae* (30%), and group II *Enterococcus* spp. (20%). The patterns of resistance to high-level aminoglycosides revealed that resistance to streptomycin was most prevalent across all species except for *E. hirae*, followed by kanamycin and coresistance to streptomycin and kanamycin. Resistance to high levels of gentamicin was observed only among isolates of *E. faecalis* and occurred only in conjunction with high-level kanamycin resistance.

Susceptibility profiles of *E. faecalis* and *E. faecium* isolates. The susceptibility of isolates to antimicrobial agents used in the food animal production environment and their human analogues was examined by using the two largest populations recovered from sampling: *E. faecalis* and *E. faecium*. No differences in susceptibility to either chloramphenicol or bacitracin were observed between *E. faecalis* and *E. faecium* isolates, with both populations susceptible to chloramphenicol and with over 90% of the MICs exceeding the highest dilution of bacitracin (Table 3). There was no overlap of MICs of bambermycin (Flavomycin) between *E. faecalis* and *E. faecium*, which had modes of 2 and >32 μg/ml, respectively. Fifty-two percent of *E. faecium* isolates were resistant to ciprofloxacin at ≥4 μg/ml, while only 1.7% of the *E. faecalis* isolates were resistant.

Both species were observed to have high resistance to tetracycline as well as a distinct separation of resistant and sensitive populations. Among the lincosamide class of antimicrobials, *E. faecalis* isolates were uniformly resistant to both clindamycin and lincomycin, while the profile of the population of *E. faecium* appeared highly resistant with more variability in MICs. Resistance to erythromycin was higher among *E. faecalis* isolates (69%) than *E. faecium* isolates (34%). Both populations were observed to have a comparatively more uniform distribution of MICs of erythromycin than those of tylosin.

Differences were also apparent among the penicillin class of antimicrobials, with 71% of *E. faecium* isolates resistant to penicillin compared with none of the isolates of *E. faecalis*. Only a single isolate of *E. faecium* was observed to be resistant to ampicillin, although 50% of the entire population of *E. faecium* had an ampicillin MIC of 8 μg/ml, one dilution less than the NCCLS breakpoint. Only seven isolates of *E. faecalis* were observed to have a quinupristin-dalfopristin MIC that was less than 4 μg/ml, while the resistance rate among *E. faecium* was 63%. Similar to the comparative distributions of clindamycin and lincomycin, the distributions of both populations were more dispersed. In particular, a bimodal distribution was observed among the MICs of the streptogramin antimicrobials for *E. faecium*: 2 and 16 μg/ml (quinupristin-dalfopristin) and 1 and 16 to 32 μg/ml (virginiamycin).

DISCUSSION

The rapid rise in antimicrobial resistance observed among human bacterial pathogens has prompted concern regarding the use of certain similar antimicrobials in both the human clinical and the food animal production environments. Analyses for determining antimicrobial resistance among targeted bacterial populations from these defined environments have often overlooked the more complex presentation of resistance to multiple antimicrobials. In this descriptive study, a population of *Enterococcus* spp. from the poultry production environment on the eastern seaboard of the United States was characterized and examined for the occurrence of coresistance among antimicrobials employed in agriculture and in human medicine.

The identification of enterococci isolated from the commercial poultry production environment did not reveal any unusual species, although eight isolates require more discriminant analysis prior to definitive identification. While multiple isolates were occasionally recovered from the same sample, the elimination of isolates with indistinguishable antibiograms from the same farm provided a collection that was conservative in its estimation of diversity but did not substantively affect the relative proportions of species isolated. The finding of *E. faecalis* predominance in this study was similar to that previously reported for poultry production environments in other parts of

TABLE 3. MIC distributions of selected antimicrobials for *E. faecalis* and *E. faecium* isolates from the poultry production environment

Class	Antimicrobial agent[a]	Range (μg/ml)	Species	No. of isolates for which the MIC (μg/ml) was[b]:												% resistant
				≤0.25	0.5	1	2	4	8	16	32	64	128	256	≥256	
Bambermycin	BMB	0.5–32	*E. faecalis*		1	43	121	10	1							NA[c]
			E. faecium							2	8	94				NA
Chloramphenicol	CHL	2–64	*E. faecalis*						170	6						0
			E. faecium					18	84	2						0
Fluoroquinolone	CIP	0.06–4	*E. faecalis*	1	23	106	43	3								2
			E. faecium	1		4	45	53	1							52
Lincosamide	CLI	0.5–2	*E. faecalis*					176								100
			E. faecium	2	8	6	1	87								85
	LIN	1–32	*E. faecalis*								22	154				NA
			E. faecium		1			2	2	4	2	93				NA
Macrolide	ERY	0.12–32	*E. faecalis*	11	2	31	10	1	5	2	2	112				69
			E. faecium	44	3	15	3	4	16	1		18				34
	TYL	1–32	*E. faecalis*		6		46	3		1	1	119				NA
			E. faecium		3		29	52	6			14				NA
Penicillin	AMP	0.25–16	*E. faecalis*	1	5	125	45									0
			E. faecium	4	2	2	14	29	52	1						1
	PEN	0.25–128	*E. faecalis*			2	121	53								0
			E. faecium	3	2		2	11	12	28	45	1				71
Peptide	BAC	8–256[d]	*E. faecalis*							1	2	9	3	1	160	NA
			E. faecium										1	7	96	NA
Streptogramin	Q-D	0.5–32	*E. faecalis*				7	17	145	7						96
			E. faecium		2	13	23	13	20	21	12					63
	VIR	0.5–32	*E. faecalis*			7		35	114	20						NA
			E. faecium	4		22	11	5	11	22	22	7				NA
Tetracycline	TET	0.25–32	*E. faecalis*		6	9	1			1	16	143				91
			E. faecium	3	17	1	1			1	7	74				79

[a] BMB, bambermycin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; LIN, lincomycin; ERY, erythromycin; TYL, tylosin; AMP, ampicillin; PEN, penicillin; BAC, bacitracin; Q-D, quinupristin-dalfopristin; VIR, virginiamycin; TET, tetracycline. Resistance breakpoints for *Enterococcus* spp. (in μg/ml) were >16 for chloramphenicol, ≥4 for ciprofloxacin, >2 for clindamycin, >4 for erythromycin, >8 for ampicillin and penicillin, ≥4 for quinupristin-dalfopristin, and >8 for tetracycline. The vertical lines indicate breakpoints for the antimicrobials.

[b] MICs which exceeded either the upper or lower limit of the tested range were listed in the next dilution series.

[c] NA, not applicable (no established NCCLS breakpoint).

[d] Expressed in international units (IU) per milliliter.

the United States (27, 40) as well as in Belgium (7), the United Kingdom (21), and Denmark (1). Studies from Japan (44), in contrast, suggested that *E. faecium* was the dominant enterococcal species of poultry fecal flora, while a Belgian study demonstrated a predominance of *E. cecorum* in older chickens (10). The variances observed with regard to species prevalence may reflect differences in isolation methodology (6), geographic disparities, or the effect of medicated feed on the intestinal enterococcal microflora (4, 27). Preliminary studies conducted in our laboratory (data not shown) suggest that the incubation temperature used during selective enrichment of samples may affect the recovery of various enterococcal species.

Phenotypic grouping based on the susceptibility to multiple antimicrobials that are frequently used in the poultry production environment provided some important observations. Most apparent is the magnitude of resistance to individual classes of antimicrobials across all isolated *Enterococcus* spp., with 98.5% resistant to the lincosamide lincomycin, 78% resistant to the streptogramin quinupristin-dalfopristin, 68% resistant to tetracycline, 54% resistant to the macrolide erythromycin, and 27% resistant to penicillin. While the indeterminate nature of group II and group III isolates and the limited data sizes of *E. avium*, *E. casseliflavus*, and *E. durans* preclude generalizations, the diversity of observed phenotypes, especially among isolates of *E. faecium* and *E. hirae*, is striking given the larger population of *E. faecalis*. Interestingly, 63% of *E. faecalis* isolates demonstrated multiresistance to lincosamide, macrolide, streptogramin, and tetracycline classes of antimicrobials, while the largest subset of *E. faecium* isolates demonstrated multiresistance to lincosamide, penicillin, streptogramin, and tetracycline antimicrobials. Acquired resistance elements that confer cross-resistance to macrolide-lincosamide-streptogramin antimicrobials have been well described for enterococci (33) and have been associated with coresistance to other drugs (20, 30, 41, 45). Isolates that express these resistance elements may be phenotypically observed as resistant to macrolide and lincosamide classes with streptogramin resistance dependent upon the presence of other resistance elements (5). However, there were individual instances of isolated lincosamide and macro-

lide resistance phenotypes as well as isolates that possessed coresistance to lincosamide and streptogramin antimicrobials in the absence of macrolide resistance.

There are few published quantitative descriptions of multiply resistant phenotypes observed among environmental *Enterococcus* spp. in the United States, especially those from the poultry production environment. Data from a Danish study illustrate the diversity of resistance phenotypes encountered among *E. faecalis* and *E. faecium* isolated from poultry as well as the frequent association of the resistance of macrolides and tetracycline with other antimicrobials (1). Streptogramin-resistant *E. faecium* from this study also appeared more likely to be resistant to tetracycline than the population of streptogramin-sensitive isolates, which is consistent with anecdotal descriptions of isolates from retail chicken from the United States, but were not more likely to be resistant to penicillin (25). Our results also suggest that streptogramin-sensitive isolates were more likely to be coresistant to macrolides.

Consistent with poultry studies from Japan (44) and Denmark (1), high-level gentamicin resistance was observed in this study only among *E. faecalis* isolates, although a Belgian report has demonstrated higher rates among *E. faecium* isolates (7). High-level gentamicin resistance was found only in isolates that also showed a high level of resistance to kanamycin, which is consistent with studies of clinical enterococci (38, 45). Similar to the observations of enterococci of poultry origin from Denmark, resistance to multiple aminoglycosides at high levels was observed among the largest populations of this study, with isolated high-level streptomycin resistance as a predominant phenotype (1). A higher prevalence of high-level streptomycin resistance was also seen among *E. faecalis* and *E. faecium* isolates of animal origin from the United States (36). Molecular studies of high-level aminoglycoside resistance among *Enterococcus* spp. suggest that phenotypic antibiogram profiles belie the tremendous diversity of mechanisms that contribute to multiresistance (22).

Resistance to the production drug bambermycin has been previously described as an intrinsic property among *E. faecium* from food or food production environments, whereas increased tolerance (MIC > 2 μg/ml) among *E. faecalis* isolates is rare (7, 12, 17). While these observations are similar to results presented here, resistance among vancomycin-resistant *E. faecium* isolates from Norway, which had not used bambermycin, does not follow this accepted pattern (2). Chloramphenicol, in contrast, is not used in the food production environment and is observed infrequently in poultry and retail meat products from the United States (17, 27), similar to observations in Japan (44) and Denmark (1). Resistance to the fluoroquinolone ciprofloxacin has not previously been recognized among enterococci from the poultry production environment in the United States, ostensibly due to a lack of interest in nonmobile resistance. Our observation of ciprofloxacin resistance among enterococcal isolates is similar to that observed among enterococci of poultry origin from The Netherlands and displays striking species differences (37).

Resistance to the lincosamide class of antimicrobials is common among enterococci and has been reported to be an intrinsic trait among enterococci with species-specific associations of resistance (12, 26). Macrolide resistance is also a frequent observation among enterococci from poultry production environments (1, 3, 21, 37, 44). This finding is not unexpected given the use of medicated feed whose ingredients (e.g., virginiamycin or tylosin) facilitate the development of resistance (2, 3, 9, 27). The association and possible horizontal transfer of this trait among enterococci, in conjunction with resistance to other antimicrobials, continue to be a source of concern (30, 41).

The prevalence of penicillin resistance among enterococci from poultry production environments in the United States is higher than that in Denmark (1), but the prevalence of ampicillin resistance is considerably lower than that observed in Japan (44) and Belgium (7). Our estimates of the prevalence of tolerance to the peptide bacitracin among the predominant enterococci from this study exceed those from Danish poultry and pig environments (1). Although in vitro studies have suggested that bacitracin use may select for vancomycin resistance by induction (23), no such association has been found in Danish poultry and pig production (4).

The prevalence of resistance to the streptogramin quinupristin-dalfopristin has been shown to increase to 100% in turkey flocks fed the analog virginiamycin (40), while the prevalence of quinupristin-dalfopristin-resistant *E. faecium* from the chicken production environment in the United States has been estimated to be between 51 and 78% (18). Those findings are consistent with our results as well as those from Denmark (1, 2, 20) but contrast with observations made in countries in which virginiamycin is not used (2). The bimodal distribution of virginiamycin MICs for *E. faecium* isolates from Japanese broilers is also consistent with our observations of streptogramin antimicrobials (44).

Resistance to tetracycline among *Enterococcus* spp. is very common, especially among those of poultry origin in the United States (27, 42) and abroad (7, 44). Tetracycline resistance has also been previously demonstrated to be linked closely to poultry production environments (37), with observations of similar distributions of MICs (43). As demonstrated by other surveys of poultry flocks (15, 35, 40) and poultry products (17) in the United States, no resistance to vancomycin was observed.

The results of this study illustrate that *Enterococcus* spp. from poultry production and processing operations in the United States are frequently resistant to multiple antimicrobials and that some of these patterns may very well reflect the use of approved antimicrobials in poultry. This work also establishes a baseline of resistance among *Enterococcus* spp. that will be useful in monitoring the dynamics of resistance longitudinally. Considering some of the current estimates of the extent of antimicrobial use in the poultry production industry for growth enhancement, the increasing potential of such an intensive agricultural operation to affect antimicrobial resistance must be weighed against the reasonable risk that treatment of human bacterial infections may be compromised.

Rising levels of resistance to multiple antimicrobials dictate the frequent and close monitoring of resistance in bacterial pathogens in both clinical and agricultural environments in the United States and abroad. Without this measure of surveillance, the management of this problem on a piecemeal basis could very well result in a further waning of the effectiveness of antimicrobials and additionally lead to a reduction of the numbers of antimicrobials available to treat human infections. The

increase in public concern has led to the ban of growth-promoting antimicrobials in the EU based on perceived risk rather than clear scientific evidence (31). Failure to exercise continuing, efficient, and sound scientific judgment in the search for a means to reduce antibiotic resistance could lead to the implementation of a similar policy in the United States.

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