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Prevalence and Antimicrobial Resistance of *Campylobacter* in Antimicrobial-Free and Conventional Pig Production Systems

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ABSTRACT

?1 The objectives of this study were to determine and compare the prevalence and antimicrobial resistance of *Campylobacter* species in swine reared in conventional and antimicrobial-free (ABF) production systems. *Campylobacter coli* was the predominant species, with 1,459 isolates (99%) in the study. We found significantly higher prevalence of *C. coli* on the ABF farms (77.3%) than on the conventional farms (27.6%) among pigs at the nursery stage ($P < 0.001$). At slaughter, we found significantly higher prevalence at the postevisceration than at the preevisceration stage ($P < 0.001$) in both production systems. The 1,459 *C. coli* isolates were tested with the agar dilution method for their susceptibility to six antimicrobials: chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, and tetracycline. Resistance was most prevalent against tetracycline (66.2% of isolates) followed by erythromycin (53.6% of isolates). Frequency of resistance to these two antimicrobials was significantly higher among conventional herds (83.4% for tetracycline and 77% for erythromycin) than among ABF herds (56.2% for tetracycline and 34.5% for erythromycin). Resistance to ciprofloxacin at the MIC (>4 mg/liter) was also found on farms in both systems. Multidrug-resistant *C. coli* strains were detected in both the conventional (7%) and ABF (4%) herds. This is the first report of ciprofloxacin-resistant strains of *C. coli* in ABF pigs in the United States. These findings highlight the high prevalence of antimicrobial-resistant *C. coli* in both conventional and ABF pig production systems and have significant implications for the persistence of antimicrobial-resistant *Campylobacter* in the pig production environment.

Foodborne diseases in the United States account for an estimated 76 million cases of illness and 5,000 deaths annually *(22). Campylobacter* is the leading cause of foodborne bacterial infection and is responsible for an estimated 2.4 million cases. Although *Campylobacter jejuni* in humans is considered to be the most important *Campylobacter* species causing infection, recent studies in Spain and the United Kingdom have highlighted the importance of *Campylobacter coli* as a human pathogen because of its resistance to various classes of antimicrobials and because it causes more indigenously acquired foodborne diseases *(35, 39).* Various animal species harbor *Campylobacter* species *(3, 9, 28, 39).* Poultry has been recognized as the primary reservoir of *C. jejuni,* and pigs are mostly implicated as reservoirs of *C. coli (17, 43). C. coli* has been suggested to be particularly suited to the swine environment and has been isolated from up to 100% of the samples collected from pigs on farms *(35).* In previous studies, the presence of *Campylobacter* has been reported on swine carcasses in the slaughterhouse at different stages of processing, with prevalence ranging from 2 to 9% at prechilling to 1.7% at the postchilling stage *(19, 25, 26).*

Foods of animal origin are the major causes of campylobacteriosis in humans *(27).* The role of pork products in causing foodborne campylobacteriosis has not been fully elucidated, even though *C. coli* has been isolated commonly from pork products in retail markets in the United States and Canada *(16, 44).* Although antimicrobials are not recommended for treating mild cases of campylobacteriosis, they are prescribed in complicated systemic cases *(1, 33).* The emergence of fluoroquinolone and macrolide resistance in *Campylobacter* species could potentiate the ability of this pathogen to disseminate widely. Resistance to important classes of antimicrobials such as the fluoroquinolones used in the treatment of severe cases of campylobacteriosis has been on the rise in the United States since 1990 *(14, 38).* Infection with fluoroquinolone-resistant strains of *Campylobacter* can prolong the duration of gastrointestinal infection compared with infection caused by susceptible strains *(14).* The role of antimicrobials used for growth promotion in animals in the development of resistance in pathogens has become an issue of debate.

The status of *Campylobacter* in swine raised in the conventional system of production where antimicrobials are used both for treatment and growth promotion has been investigated previously *(30, 35, 41).* However, there is paucity of information as to the comparative significance of *Campylobacter* occurrence and antimicrobial resistance among pigs reared in antibiotic-free (ABF) and conventional production systems. Studies comparing these two production systems have been conducted with other species such as poultry and dairy cows in the United States *(18, 37).* The present study was designed to determine and compare the prevalence and antimicrobial susceptibility of *Campylobacter* in conventional and ABF pig production systems on the farm and at slaughter.

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MATERIALS AND METHODS

Sample collection. Samples were collected in North Carolina on the farm and in slaughter areas of processing plants from swine reared in two production systems: the conventional and ABF systems. The two kinds of farms included in the study were geographically distant, and all except one ABF farm were located in the eastern part of the state. Under the conventional system of raising pigs, antimicrobials were used as feed additives both for growth promotion and for treatment purposes. Information on antimicrobial use was collected from swine producers. Oxytetracycline (dose rate of 400 g/ton) and tylosin (Tylan, dose rate of 40 g/ton) were added to the feed at the nursery and finishing farms. Injectable penicillin and ceftiofur also were given at both the nursery and finishing stages. In the ABF type production system, antimicrobials were not used for growth promotion or for treatment after the weaning stage (3 weeks of age). Any ABF pig that had to be treated with antimicrobials for an infection was immediately removed from the group.

A total of 21 groups of pigs were included in this study, and samples were collected for 2 years, from October 2002 to October 2004. At the farm, fecal samples were collected from pigs at nursery farms (6 to 8 weeks of age) and finishing farms (within 48 h of marketing). Pigs sampled during the study were ear tagged and tattooed for individual identification at subsequent stages of processing at the slaughter plant. Approximately 30 pigs were included in each sample group. Seven group of pigs (three groups from conventional farms and four from ABF farms) were sampled at both the nursery and finishing farms. We also collected fecal samples from 14 additional groups of pigs (eight conventional and six ABF) at the finishing farms (within 48 h of marketing). Carcass swabs from all 21 groups of pigs were subsequently obtained at the slaughter plant at three stages of processing: preevisceration (immediately before evisceration of the gut), postevisceration (after gut evisceration), and postchill (after the carcass was chilled and ready for packing). At every farm visit, approximately 10 g of fresh fecal sample was collected with a gloved hand directly from the rectum of each pig. Fecal samples were transported to the laboratory on ice and processed for *Campylobacter* on the same day as arrival at the laboratory.

Slaughter samples (carcass swabs) were collected from two slaughter plants where all 21 groups of pigs were processed. The first slaughter plant processed both the conventional and ABF pigs and used a blast chiller (−30°C for 2 h) for rapid cooling of carcasses. The ABF pigs in this plant were processed only on the first day of every week and only during the first shift to prevent cross-contamination from conventionally reared pigs. Sixteen groups of pigs (11 conventional and five ABF) were processed in the first plant. This plant was also cleaned and disinfected every weekend to prevent contamination of carcasses. The second slaughter plant processed only ABF pigs and used overnight chilling of the carcasses (1 to 4°C for approximately 18 h). The remaining five ABF groups were processed at the second plant. Sterile swabs soaked in 10 ml of buffered peptone water (Becton Dickinson, Sparks, Md.) were swiped along the midline of the carcass extending from the jowl to the ham. One sample swab was collected from each carcass, and a total of 10 carcasses per group were swabbed at each of the pre- and postevisceration stages. At the postchill stage, we collected samples from 10 carcasses per group and two swab samples from each carcass. The method recommended by the U.S. Department of Agriculture (USDA) *(29)* was used on one side, and the single-swipe method was used on the other side to generate baseline data on whether the two methods provide comparable results. This design resulted in a total of 40 samples from 30 carcasses from each group of pigs at the slaughter plants, 20 samples from the 10 carcasses each at pre- and postevisceration stages, and another 20 samples from the 10 carcasses at the postchill stage. Samples were transported to the laboratory on ice and processed on the same day upon arrival.

***Campylobacter* isolation.** Fecal sample from the farms were directly plated (loopful, approximately 10 μl) onto campy-cefex selective plates *(31)* and incubated under microaerobic conditions (10% CO_2, 5% O_2, and 85%N_2) with Anaeropacks (Remel, Lenexa, Kans.) at 42°C for 48 h. All the incubations in subsequent steps were carried out under microaerobic conditions at the same temperature and duration unless stated otherwise. Carcass swabs were soaked in 30 ml of Bolton broth (Oxoid, Hampshire, UK) and incubated for 48 h. Swabs in each Whirl-Pak bag were then squeezed, and a loopful of enriched liquid was aseptically withdrawn and streaked onto campy-cefex plates and incubated. Three *Campylobacter* colonies growing on the campy-cefex plate from each presumptive positive sample (fecal or carcass sample) were tested biochemically using the catalase test (3% H_2O_2, release of oxygen indicated by bubble formation) and the oxidase test (tetramethyl-*p*-phenylenediamine, color change of colonies) (Becton Dickinson) for confirmation. Colonies that were positive in both the catalase and oxidase tests were streaked onto Mueller-Hinton agar plates (Remel) and further identified to species with a PCR assay. Individual *Campylobacter* isolates with appropriate database numbers were stored at −80°C in brain heart infusion broth (Becton Dickinson) supplemented with 35% dimethyl sulfoxide (Sigma, St. Louis, Mo.) until further analysis.

***Campylobacter* species determination.** We used species-specific primers for PCR identification of important species of *Campylobacter*, particularly *C. coli* and *C. jejuni*. The *ceuE* gene that encodes a protein involved in siderophore transport was used for detection of *C. coli*, and the hippuricase gene (*hipO*) was used for detecting *C. jejuni (13, 15).* DNA was purified from freshly grown cultures with the DNeasy Tissue kit (Qiagen, Valencia, Calif.). The forward and reverse primers for *ceuE* gene amplification were CC2 (5′-GATTTTATTATTTGTAGCAGCG-3′) and CC3 (5′-TCCATGCCCTAAGACTTAACG-3′) *(13)*, and those for *hipO* gene amplification were Hip1A (5′-ATGATGGCTTCTTCGGATAG-3′) and Hip2B (5′-GCTCCTATGCTTACAACTGC-3′) *(15).* PCR conditions were initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min, and final extension at 72°C for 7 min. Reactions were maintained at 4°C until amplicons were separated by electrophoresis on 1.5% agarose gels and stained with ethidium bromide.

Antimicrobial susceptibility testing. The agar dilution method as recommended by the Clinical and Laboratory Standards Institute (formerly the NCCLS) subcommittee on veterinary antimicrobial susceptibility testing was used to determine the resistance and susceptibility of *Campylobacter* strains *(11).* We tested the isolates for their susceptibility against a panel of six antimicrobials: chloramphenicol (Ch; 0.25 to 128 mg/liter), ciprofloxacin (Cip; 0.008 to 4 mg/liter), erythromycin (Ery; 0.06 to 32 mg/liter), gentamicin (Gen; 0.06 to 32 mg/liter), nalidixic acid (Nal; 0.25 to 128 mg/liter), and tetracycline (Tet; 0.06 to 32 mg/liter) *(11).* All the antimicrobials were procured from Sigma except ciprofloxacin (Serologicals Proteins, Kankakee, Ill.). The CLSI breakpoint interpretative criteria for *Enterobacteriaceae* were used for all the antimicrobials except erythromycin because the interpretive standard breakpoint levels for the *Campylobacteriaceae* are not yet available *(24).* For erythromycin (8 mg/liter), the breakpoint level used by the National Antimicrobial Resistance Monitoring System

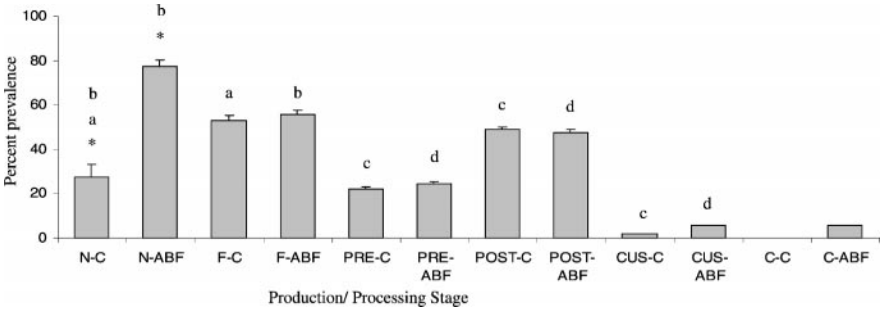

FIGURE 1. C. coli *prevalence at the farm and at slaughter in pigs produced in two production systems. Abbreviations (number of pigs or carcasses sampled): N-C, conventional nursery (105); N-ABF, ABF nursery (141); F-C, conventional finishing (370); F-ABF, ABF finishing (292); PRE-C, conventional preevisceration (103); PRE-ABF, ABF preevisceration (78); POST-C, conventional postevisceration (98); POST-ABF, ABF postevisceration (88); CUS-C, conventional postchill, USDA method (107); CUS-ABF, ABF postchill, USDA method (88); C-C, conventional postchill, single-swipe method (108); C-ABF, ABF postchill, single-swipe method (87). Bars with the same superior letters are significantly different (*P $<$ *0.05).*

was adopted *(40). C. jejuni* ATCC 33560 was used as the quality control organism for this test *(11).* The MIC_{50} breakpoints used for each antimicrobial were 32 mg/liter for chloramphenicol, 4 mg/liter for ciprofloxacin, 8 mg/liter for erythromycin, 16 mg/liter for gentamicin, 32 mg/liter for nalidixic acid, and 16 mg/liter for tetracycline.

C. coli isolates were streaked on Mueller-Hinton agar plates supplemented with sheep blood and incubated under microaerophilic conditions for 48 h. A loopful of fresh culture was diluted in 3 ml of Mueller-Hinton broth to a concentration of 0.5 McFarland turbidity standards (approximately 10^8 CFU/ml) as determined with a colorimeter (bioMérieux, Hazelwood, Mo.). Twofold serial dilutions of the antimicrobials were made in sterile distilled water in the appropriate dilution range. One milliliter of the diluted antimicrobial in 2 ml of sheep blood was added to 17 ml of Mueller-Hinton agar making a total of 20 ml agar medium with the desired concentration of the antimicrobial. The diluted cultures (approximately 10^4 CFU per inoculum) were then plated onto the antimicrobial plates with a Cathra replicator with 1-mm-diameter pins (Oxoid, Inc., Ottawa, Ontario, Canada). The plates were then incubated at 42°C for 24 h, and the MIC was recorded for each antimicrobial. Resistance to each antimicrobial was determined from the recommended breakpoints. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobials.

Statistical analysis. *Campylobacter* prevalence, frequency of antimicrobial resistance profiles, and patterns between and within the conventional and ABF production systems at the farm and at slaughter were compared using the chi-square test (Minitab, Inc., State College, Pa.) and Fisher's exact two-tailed test *(20)* wherever applicable. Differences were considered significant at $P < 0.05$.

RESULTS

***Campylobacter* prevalence at farm and at slaughter.** To determine the prevalence and antimicrobial resistance profile of *Campylobacter* species in swine raised in two different production systems, 908 pigs and 562 carcasses from 21 pig groups were sampled at farms and at slaughter plants in North Carolina. Of the 1,634 *Campylobacter* isolates recovered, 1,472 isolates (1,117 on the farm and 355 at slaughter) were identified to species with species-specific PCR assays. The remaining 162 *Campylobacter* isolates could not be cultured despite multiple attempts. *C. coli* accounted for 99% (1,459) of these isolates. None of the remaining 13 isolates were *C. jejuni* and were not included in the subsequent analyses.

Comparison of the two production systems at the nursery farm revealed significantly higher prevalence of *C. coli* ($P < 0.001$) in pigs on the ABF farms (77.3%) than on the conventional farms (27.6%) (Fig. 1). This higher prevalence was mainly attributed to the difference in prevalence at the nursery farms and at processing (slaughter), because no significant difference in the prevalence of this pathogen between the two systems was detected at the finishing farm (53 and 55.8% for ABF and conventional farms, respectively). At the slaughter stage, there was a significantly higher recovery of *Campylobacter* at postevisceration than at preevisceration. Chilling significantly reduced the recovery of *Campylobacter* in all groups ($P < 0.002$). The USDA and the single-swipe carcass swabbing methods resulted in similar *Campylobacter* recovery in the postchill samples ($n = 195$); 3.6 and 2.6% of the swabs were positive for *Campylobacter* with the USDA and single-swipe methods, respectively. At the two slaughter plants, significantly more *C. coli* isolates were recovered from carcasses that had been chilled overnight than from carcasses that had been blast chilled for 2 h and then chilled overnight ($P < 0.001$).

Antimicrobial resistance of *Campylobacter* isolates. We compared the distribution of antimicrobial-resistant *C. coli* isolates between and within the two production systems. Regardless of the production system and production stage, *C. coli* isolates exhibited highest resistance against tetracycline (66.2%) and erythromycin (53.6%) (Table 1). A significantly higher percentage of tetracycline- and erythromycin-resistant *C. coli* isolates were detected within the conventional system than within the ABF system ($P < 0.05$), supporting the association between antimicrobial use and development of resistance. Within the conventional system, resistance to tetracycline and erythromycin was common in isolates from both the farm and the slaughter plant. However, in the ABF system, significantly higher fre-

TABLE 1. *MIC data and antimicrobial resistance profiles of* C. coli *isolates from the two swine production systems at different stages*

Antimicrobial[a]	Dilution (mg/liter)[b]	Breakpoint (mg/liter)[c]	Production stage[d]	Production system	% of isolates with MIC (mg/liter) of:											No. (%) of resistant isolates
					≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥32	
Ch	0.25–128	32	Farm	Conventional			0.4	1.5	0.6	19.7	54.6	16.8	4.2	1.5[e]	0.2	8 (1.7)
				ABF				1	1	12.4	47.5	35	1.5	0.9[e]	0.5	9 (1.4)
			Slaughter	Conventional					1	19.4	64.8	8.7	1.6	0.5[e]	3.8	8 (4.3)
				ABF			0.6	2.4	2.4	16.2	66.2	10.8		0.6[e]	0.6	2 (1.2)
Cip	0.008–4	4	Farm	Conventional	15.7	40	34.8	5.3	0.6	0.4	2.8[e]					13 (2.8)
				ABF	12.9	41	28.1	11.3	3	2.7	0.6[e]					4 (0.6)
			Slaughter	Conventional	17.4	39.9	39.3	0.5	2.7							0
				ABF	50	40.9	3	3	1.2							0
Ery	0.06–32	8	Farm	Conventional	0.2		0.2	1.1	4.2	6.7	10.4	3.5[e]	1.5	3.1	68.9	347 (77)
				ABF	0.3		2.3	5.4	14	28.3	15.3	5.8[e]	3.1	2.2	22.2	228 (34.5)
			Slaughter	Conventional				1.6	3.3	7.7	6.6	3.3[e]	2.7	3.3	69.8	149 (81.4)
				ABF		1.2	0.6	6	11.4	27.7	13.8	12[e]	5.4	2.4	16.8	67 (40.4)
Gen	0.06–32	16	Farm	Conventional	0.6	0.4	1.3	21.5	50	22.9	1.3	0.4	0.4[e]		0.4	4 (0.8)
				ABF	0.3	1	2.1	17.5	48	27.2	3.2	0.4				0
			Slaughter	Conventional			6	27.3	56.8	9.8				0.5		1 (0.5)
				ABF		0.6	1.2	34.3	46.3	16.2		0.6	0.6[e]			1 (0.6)
Nal	0.25–128	32	Farm	Conventional					0.6	5.5	27.1	48.2	11.1	2[e]	5.3	33 (7.3)
				ABF			0.5	0.3		6.5	32	44	8.9	2.2[e]	5.5	54 (8.4)
			Slaughter	Conventional						9.3	43.7	38.79	6.5	0.5[e]	0.5	3 (1.5)
				ABF			0.6	0.6	0.6	16.26	57.8	20.4	3		0.6	1 (0.6)
Tet	0.06–32	16	Farm	Conventional		0.4	1.3	0.6	1.1	3.3	0.4	9.3	6.7[e]	21.8	54.9	375 (83.4)
				ABF	0.6	1.8	4.8	4.7	5.5	11.5	8.5	7	6.8[e]	11.8	37.2	373 (56.2)
			Slaughter	Conventional			0.5	0.5			12.6	6.5	4.9[e]	16.9	57.9	147 (80.8)
				ABF		6.6	4.2	7.2	1.8	4.2	21	9.6	3.6[e]	19.2	22.8	61 (36.6)

[a] Ch, chloramphenicol; Cip, ciprofloxacin; Ery, erythromycin; Gen, gentamicin; Nal, nalidixic acid; Tet, tetracycline.
[b] Dilution range based on the approved CLSI standards for *Campylobacter*.
[c] Breakpoint based on *C. jejuni* ATCC 33560.
[d] Number of *C. coli* isolates recovered from farms: 450 on conventional farms and 660 on ABF (antimicrobial-free) farms. Number of *C. coli* isolates recovered at slaughter plants: 183 from conventional pigs and 166 from ABF pigs.
[e] Breakpoint.

quency of resistance to tetracycline was observed in isolates from the farms (56.3%) than in isolates from the slaughter plants (36.6%) ($P < 0.001$).

Resistance to ciprofloxacin was detected in *C. coli* isolates from on-farm specimens from both the conventional (2.8%) and ABF (0.6%) herds (total $n = 17$). All the ciprofloxacin-resistant isolates were also resistant to nalidixic acid. Gentamicin- and chloramphenicol-resistant isolates were also observed in 0.4 and 1.8%, respectively, of the total isolates tested.

Antimicrobial resistance patterns of *Campylobacter* isolates. Overall, we observed 20 different resistance patterns, including a pansusceptible pattern exhibited by 1,152 (78.9%) of the isolates (Table 2). Fifteen of these patterns are listed in Table 2, and the remaining 5 patterns were exhibited by single isolate each and were not included in the table. A significantly higher proportion of isolates from the ABF system (33%) at the farm and slaughter were pansusceptible compared with isolates from the conventional system (5.2%) ($P < 0.001$). Ery-Tet was the most common resistance pattern regardless of the type of production system. However, a higher proportion of isolates from finishing farms (60.6 and 21% for conventional and ABF farms, respectively) exhibited the Ery-Tet pattern than did those from nursery farms (47 and 15% for conventional and ABF farms, respectively). There were 11 different MDR patterns among 79 (5.4%) of the isolates; the most common was Ery-Nal-Tet ($n = 40$, 2.7%). *C. coli* isolates from the conventional system, both on the farm and at slaughter, more often exhibited MDR than did isolates from the ABF system ($P = 0.005$). Fewer MDR patterns were found in isolates at slaughter than on the farm: Cip-Gen-Nal-Tet ($n = 1$), Ch-Ery-Gen ($n = 1$), Ch-Ery-Nal-Tet ($n = 1$), and Ch-Ery-Nal ($n = 1$).

MIC values across the two production systems. MIC values were analyzed to determine whether there was variation in the MIC among resistant isolates based on the established breakpoints. Such comparison, however, may not be conclusive because the strains may exhibit a one-dilution difference in MIC even though they are clonal. The antimicrobial of special interest in the MIC analysis was chloramphenicol. Although comparable frequency of resistance to chloramphenicol was observed for *C. coli* isolates both on the farm ($n = 17$) and at slaughter ($n = 10$), isolates from the slaughter plants were resistant to chloramphenicol at a fourfold higher MIC (128 mg/liter) than were isolates from the farms ($P < 0.001$), indicating that different chloramphenicol-resistant strains may be recovered at different stages of the production continuum. Isolates from the same pig (Ch-Cip-Ery-Nal-Tet pattern, MIC = 32 mg/liter) were clustered in one group as determined by pulsed-field gel electrophoresis (data not shown), and the third isolate (Ch-Ery-Tet pattern, MIC = 64 mg/liter) clustered in a separate group. Although the three isolates genotyped may not be representative, the result indicate that differences at the phenotypic level (MIC values) can be corroborated with a genotypic approach. No variation in MIC has been detected for tetracycline, the antimicrobial to which resistance was most common. Except for isolates from the ABF slaughter plant, which were mostly susceptible at an MIC of 4 mg/liter (20%), resistant isolates from both the production systems exhibited an MIC of 32 mg/liter for tetracycline at all stages of production. However, variation in MIC was found for erythromycin, the second most commonly resisted antimicrobial. Most of the isolates from the conventional system (farm, 68.9%; slaughter, 71.9%) were resistant to erythromycin, even at the highest concentration of 32 mg/liter. Isolates from the ABF system (farm, 28.3%; slaughter, 27.6%) were mostly grouped at an MIC of 2 mg/liter, exhibiting a 16-fold reduction in MIC compared with isolates from the conventional system. All ciprofloxacin-resistant isolates ($n = 17$) exhibited resistance to the highest concentration (4 mg/liter) tested in this study. Resistance to nalidixic acid at the maximum concentration of 128 mg/liter was more common on the ABF farms (3.3%) that on the conventional farms (1.1%).

DISCUSSION

Campylobacter has been reported from pigs on farms and from pig carcasses in slaughter plants in many studies *(5, 16, 17, 25, 30, 31, 41).* However, these studies have been restricted to pigs reared under the conventional system, in which antimicrobials are routinely used for treatment and growth promotion. There is paucity of information on the prevalence and antimicrobial resistance of this pathogen on ABF swine farms. This study was conducted with the primary objective of determining and comparing the prevalence and antimicrobial resistance profile of *Campylobacter* strains isolated from pigs in the conventional and ABF productions systems.

In previous studies, pigs have repeatedly been implicated as important carriers of *C. coli.* In conventional systems, higher prevalence of *C. coli* has been reported, ranging from 57.8% in newborn piglets to 100% in adult pigs *(35, 43).* Consistent with previous reports, *C. coli* was the predominant *Campylobacter* species isolated from 99% of the total samples cultured. We found high prevalence of *C. coli* in both ABF and conventional herds. This finding further emphasizes the importance of pigs as reservoirs of this pathogen regardless of antimicrobial use in the production environment. In previous studies conducted in the broiler industry, a significant difference between the two production systems was reported, with higher prevalence of this pathogen in the ABF system *(2, 18).* In a study of dairy cattle, no significant difference in the prevalence of *C. coli* was reported between the organic herds (those in which no antimicrobials were used at any stage of production) and the conventional herds *(37).* Decrease in the carriage of *Campylobacter* with age, as seen in the ABF system in this study, has been reported previously in pigs and dogs *(21, 42, 43).* At the slaughter plant, we observed significant increases in the prevalence at the postevisceration stage followed by a significant decrease in the postchill stage. The significant increase in recovery of *Campylobacter* from postevisceration swabs suggests the impact of various manipulations during and after evisceration, including potential gut spillage, cross-contamination, and other external

TABLE 2. *Antimicrobial resistance pattern[a] observed among* C. coli *isolates at the farm and at slaughter for the two production systems[b]*

	Farm				Slaughter					
	Nursery		Finishing		Preevisceration		Postevisceration		Postchill (USDA)	
Resistance patterns[c]	Conv	ABF	Conv	ABF	Conv	ABF	Conv	ABF	Conv	ABF
Pansusceptible	1 (1.6)	81 (27.5)	26 (6.7)	132 (35.6)	1 (1.5)	28 (51.9)	5 (4.3)	23 (23.5)	0	7 (58.3)
Ch	0	0	0	3 (0.8)	0	0	0	0	0	0
Ery	3 (4.7)	21 (7.1)	46 (12)	46 (12.4)	27 (41.5)	8 (14.9)	4 (3.5)	17 (17.4)	0	2 (16.8)
Nal	0	2	0	2 (0.5)	0	0	0	0	0	0
Tet	11 (17.2)	125 (42.4)	63 (16.3)	77 (20.8)	1 (1.5)	13 (24)	28 (24.4)	24 (24.5)	0	2 (16.8)
Ch-Ery	0	0	0	3 (0.8)	0	0	0	0	0	0
Ery-Tet	30 (47)	44 (15)	232 (60)	78 (21)	32 (49.2)	5 (9.3)	70 (60.1)	32 (32.7)	5 (100)	1 (8.3)
Nal-Tet	1 (1.6)	10 (3.4)	2 (0.5)	8 (2.2)	0	0	0	0	0	0
Ch-Ery-Tet	1 (1.6)	1 (0.3)	3 (0.8)	2 (0.5)	3 (4.6)	0	7 (6)	0	0	0
Cip-Nal-Tet	0	1 (0.3)	0	2 (0.5)	0	0	0	0	0	0
Ery-Nal-Tet	3 (4.7)	9 (3)	12 (3.1)	16 (4.3)	0	0	0	0	0	0
Cip-Ery-Nal-Tet	10 (15.6)	0	0	1 (0.3)	0	0	0	0	0	0
Ch-Cip-Ery-Nal-Tet	0	0	2 (0.5)	0	0	0	0	0	0	0
Ery-Gen-Tet	3 (4.7)	0	0	0	0	0	0	0	0	0
Ery-Nal	0	1 (0.3)	0	2 (0.5)	0	0	0	0	0	0

[a] Number (%) of *C. coli* isolates showing the resistance pattern.

[b] Conv, conventional system; ABF, antimicrobial-free system.

[c] Ch, chloramphenicol; Cip, ciprofloxacin; Ery, erythromycin; Gen, gentamicin; Nal, nalidixic acid; Tet, tetracycline. Five resistance patterns not shown were exhibited by a single *C. coli* isolate each: Cip-Ery-Gen-Nal-Tet (conventional; nursery), Ch-Ery-Nal (conventional, preevisceration), Cip-Gen-Nal-Tet (conventional, postevisceration), Ch-Ery-Gen (ABF, postevisceration), and Ch-Ery-Nal-Tet (ABF, postevisceration). Number of isolates tested per production system is given in Table 1.

factors. In studies done previously in slaughter plants, *Campylobacter* prevalence ranged from 2 to 9% on pig carcasses after evisceration *(19, 26, 31).* Significant reduction seen at the postchill stage in both production systems was expected because *Campylobacter* is highly susceptible to cold and dry conditions *(5, 26).* In an experimental study conducted by Chang et al. *(5),* the blast chilling method was more effective than the conventional chilling method for significantly reducing *C. coli, Salmonella* Typhimurium, and *Listeria monocytogenes* on pork carcasses. Comparison of two carcass swabbing methods, the USDA and single-swipe methods, revealed no difference in recovery of *C. coli* from carcasses at the postchill stage. We recommend conducting a more thorough study of these two methods with a larger sample size to obtain corroborating results.

Antimicrobial resistance was most commonly found against tetracycline and erythromycin (Table 2), similar to findings in other studies *(7, 30, 32, 41).* There was a significant difference in resistance to these antimicrobials between the two production systems. Higher frequency of resistance was detected in conventional herds than in ABF herds, consistent with the association between antimicrobial use and resistance. Chlortetracycline and the macrolide tylosin are the two most commonly used antimicrobials for growth promotion in the conventional swine production system *(23).* On the conventional farms sampled, oxytetracycline and Tylan (tylosin) were used in feed for growth promotion at the nursery and finishing stages. The absence of antimicrobial selective pressure in the ABF system could explain the lower proportion of resistant *C. coli* isolates. Although the frequency of resistance for these two antimicrobials was relatively lower in ABF herds, a high proportion of the isolates from the ABF herds were resistant to both tetracycline and erythromycin (56.2 and 36.6% for Tet and 34.5 and 40.4% for Ery on the farm and at slaughter, respectively). A significantly higher prevalence of tetracycline resistance on the farm compared with at slaughter suggests that different sources, particularly environmental sources, may be transmitting these resistant strains on the farm. In previous studies, the temporal relationship between use of antimicrobials and emergence of antimicrobial-resistant strains of pathogens has been reported *(10, 14, 28).* In similar studies comparing the two production systems in broilers, significantly higher resistance to tetracycline and erythromycin was reported for the conventional than for the organic production system *(2, 8, 18).*

Resistance to erythromycin is of concerning because macrolide drugs are often chosen (in addition to ciprofloxacin) for treating severe cases of campylobacteriosis in humans *(36).* Comparison of the MIC values for both of these antimicrobials in the two systems revealed that isolates from the ABF system had a 16-fold lower MIC than did their counterparts from the conventional system. Desmonts et al. *(8)* reported similar results in broilers; a majority of the *C. coli* isolates from the free-range broilers (antimicrobial free) had lower MICs for erythromycin than did those from the conventionally reared broilers.

Resistance to the fluoroquinolone ciprofloxacin was also detected at the farm in both the conventional and the ABF production systems. This finding is very important because ciprofloxacin-resistant *C. coli* has not been reported previously from ABF pigs in the United States, and no fluoroquinolone antimicrobial use has been reported for either of the two production systems. Therefore, detection of these resistant strains may indicate the possible role of environmental cross-contamination via other risk factors such as exposure to other reservoir animals, including humans. Ciprofloxacin-resistant strains have been reported in 14, 17, and 100% of the *C. coli* strains in previous studies conducted outside the United States *(4, 35, 41).* The relatively lower number of isolates exhibiting resistance to chloramphenicol and gentamicin is in agreement with findings from other studies; no resistance to either of these antimicrobials was found in *C. coli* isolates from pork *(13),* and low resistance to gentamicin (0 and 3.3%) was found in isolates from pigs *(4, 41).* Resistance to chloramphenicol is noteworthy because use of this antimicrobial has not been reported in the United States in the last two decades. The Ery-Nal-Tet resistance pattern was the most common MDR pattern and has been reported previously by Payot et al. *(30)* as the most common MDR pattern in *C. coli* isolates. In previous studies, MDR strains of *C. coli* have been found in different parts of the world *(4, 6, 30, 34, 41).*

The results of this study highlight the common occurrence of antimicrobial-resistant *C. coli* both on the farm and at slaughter in pigs from both conventional and ABF systems. Although we detected higher numbers of resistant and MDR isolates in pigs from the conventional system, the high proportion of antimicrobial-resistant *C. coli* isolates in pigs from the ABF system warrants concern and points to the possible role of other environmental factors, in addition to direct antimicrobial use, in resistance development and transmission. The detection of ciprofloxacin-resistant *C. coli* isolates in pigs also is of concern because this antimicrobial is not used in swine production and is the primary antimicrobial used in treatment of severe invasive cases of campylobacteriosis in humans.

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