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Longitudinal Study of the Persistence of Antimicrobial-Resistant *Campylobacter* Strains in Distinct Swine Production Systems on Farms, at Slaughter, and in the Environment

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The objectives of this study were to compare and characterize the prevalence of antimicrobial-resistant (AR) *Campylobacter* in conventional and antimicrobial-free (ABF) production systems on farms, at slaughter, and in the environment. Fecal and environmental samples were collected from ABF farms (pigs, 1,239; environment, 797) and conventional farms (pigs, 1,650; environment, 1,325). At slaughter, we collected samples from carcasses, including postevisceration swabs, postchill swabs, and mesenteric lymph nodes from ABF systems (postevisceration swabs, 182; postchill swabs, 199; mesenteric lymph nodes, 184) and conventional systems (postevisceration swabs, 272; postchill swabs, 271; mesenteric lymph nodes, 255) at separate processing facilities. We also sampled the processing plant environment, including truck and lairage floor swab samples (ABF, 115; conventional, 90). Overall, a total of 2,908 *Campylobacter* isolates, including *Campylobacter coli* (farm, 2,557, 99.8%; slaughter, 341, 98.3%) and *Campylobacter jejuni* (farm, 4, 0.2%; slaughter, 6, 1.7%), were isolated in the study. There was no significant difference in the prevalence of *Campylobacter* between ABF and conventionally raised pigs (farrowing, $P = 0.20$; nursery, $P = 0.06$; finishing, $P = 0.24$) and the environment ($P = 0.37$). At slaughter, *Campylobacter* was isolated from all of the stages, including postchill. The highest frequencies of resistance were exhibited against tetracycline (ABF, 48.2%; conventional, 88.3%). Ciprofloxacin-resistant *C. coli* isolates were observed in conventionally raised (17.1%) and ABF (1.2%) pigs ($P = 0.11$). Antimicrobial use data from conventional farms indicated significant associations between oxytetracycline use and tetracycline resistance in the nursery pigs ($P = 0.01$), between tiamulin exposure and azithromycin and erythromycin resistance in nursery ($P < 0.01$) and finishing ($P < 0.01$) pigs, and between enrofloxacin exposure and ciprofloxacin and nalidixic acid resistance in farrowing ($P < 0.01$) and nursery ($P < 0.01$) pigs. Identical antimicrobial resistance profiles were observed in the pigs and their environments on farms and at slaughter. In summary, our results highlight the persistence and dissemination of AR *Campylobacter* from farm to slaughter in ABF and conventionally raised pigs and their environments.

According to the Centers for Disease Control and Prevention (CDC), *Campylobacter* is the second most important bacterial food-borne pathogen after *Salmonella* causing food-borne illnesses in the United States (24). *Campylobacter jejuni* is considered to be the species primarily responsible for disease in humans (26); however, *Campylobacter coli* cases are frequently under-reported, and thus its true public health impact is unknown (16, 30). In 2000, *C. coli* was estimated to be responsible for more than 25,000 cases in the United Kingdom (30). Foods of animal origin are one of the most important sources of *Campylobacter* infections, with sporadic cases being more commonly observed than outbreaks (14, 37). In pigs, *C. coli* was the predominant species reported in previous studies (15, 31). The routine use of antimicrobials in conventional or intensive swine production has been attributed to the emergence of antimicrobial-resistant (AR) bacterial pathogens (1), some of which have a public health impact. *C. coli* in pigs (15, 31) is recognized for its high frequency of resistance to antimicrobials, including macrolides (azithromycin and erythromycin), quinolones (ciprofloxacin and nalidixic acid), and tetracycline (22, 25, 29).

Public health concerns owing to the emergence and dissemination of AR bacterial pathogens from commercial food animals, in addition to other issues related to health, environment, and animal welfare, have led to an increase in the demand of niche markets, including antimicrobial-free (ABF) and organic products over the last decade (33). Nationally, there was a 58% increase in the number of certified organic hogs and pigs between 2000 and 2005 (12), while the total certified organic livestock experienced a 31% increase from 2007 to 2008 (34). Interestingly, data from organic and ABF cattle, poultry, and swine indicate that AR bacterial pathogens, including *Salmonella* and *Campylobacter* spp., are prevalent in these alternative production systems (19, 21, 29, 32). This highlights a potential role played by environmental reservoirs in the transmission of these AR strains to animals that needs to be identified. There is no information regarding the possible sources that aid in the transmission of AR *Campylobacter* to ABF swine. We also do not know how the outdoor production environment affects the phenotypic diversity of *Campylobacter* on farms and at slaughter or how different or similar it is compared to the environment for indoor commercially raised pigs. To answer these questions, we conducted a longitudinal based study to determine *Campylobacter* prevalence and the AR phenotype in ABF and conventional swine production systems on farms and at slaughter. In addition, the role played by the environment in the transmission of AR *Campylobacter* to pigs at different stages of production was examined.

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MATERIALS AND METHODS

A total of 10 cohorts of conventionally raised pigs and 8 cohorts of ABF pigs (35 pigs/cohort) were sampled five times each on 30 conventional and 8 ABF farms. The conventionally raised pigs belonged to two different production companies, while the ABF farms were owned by individual producers. Sampling was completed in a 2-year period from October 2008 to December 2010 in North Carolina. The sample size was calculated according to type I ($\alpha = 0.05$) and type II ($\beta = 0.20$) error rates. We estimated that 27 to 35 pigs would be needed to detect a statistically significant difference in the proportion of *Campylobacter*-positive pigs in the two production systems. Healthy pigs were purposely selected in the present study to ensure sampling at slaughter. Differences in the number of pigs sampled at the three swine production stages in conventional and ABF farms were observed due to pig mortality. Pigs reared under the conventional farms were housed indoors under an all-in all-out (AIAO) system and received antimicrobials for therapeutic and prophylaxis purposes. These pigs were part of a three-site rearing system and therefore moved from one location to another. Samples from ABF and conventionally raised pigs were collected from the farms at five time points, including once at the farrowing stage (sows and 7- to 10-day-old piglets), twice at the nursery stage (4 and 7 weeks old), and twice again at the finishing stage (16 and 26 weeks of age). The final finishing sampling at 26 weeks of age was done 48 h before the pigs were transported to the processing facility. Under the ABF production system, pigs were housed outdoors in the open under a continuous flow and completed all of their production stages on the same site. ABF systems did not use antimicrobials for any purpose. Any outdoor pig that was treated for infection with antimicrobials lost its ABF status and was removed from the herd. Pigs and environment at farm and slaughter were sampled multiple times during the study to determine the phenotypic diversity of *Campylobacter* at different production stages and whether pathogen profile changes in pigs is also reciprocated in the environment.

Farm and environmental sampling. On the farms, samples were collected from pigs and their environment in ABF (pigs, 1,239; environment, 797) and conventional (pigs, 1,650; environment, 1,325) systems. Fresh fecal samples from 35 healthy pigs per farm and corresponding environmental samples were collected at different stages of production. The piglets at farrowing were ear tagged to allow proper identification and sampling of the same group of pigs at subsequent sampling stages. Sterile fecal loops (Webster Veterinary, Devens, MA) were used for the collection of feces from piglets, while nursery and finishing pigs were sampled with gloved hands. The environmental samples collected at ABF and conventional farms included feed (ABF, 200; conventional, 250), water (ABF, 198; conventional, 250), soil (ABF, 199; conventional, 250), and drag swab (ABF, 200; conventional, 250) samples of the floor and structures. All of the ABF farm environmental samples, including water, feed, soil, and drag swabs, were collected outdoors. On conventional farms, the environmental samples were collected indoors, except for soil samples, which were collected outside the barns. In addition, at conventional farms, we also collected lagoon samples ($n = 245$) and floor swab samples from the trucks ($n = 80$) that moved the pigs from one farm stage to the other along the production line. Swabs used for the collection of environmental samples were premoistened with 10 ml of buffered peptone water (Becton Dickinson, Sparks, MD). The environmental samples were placed in sterile Whirl-Pack bags (Nasco, Fort Atkinson, WI), except for the water and lagoon samples, which were collected in sterile cups (VWR, Suwanee, GA).

Slaughter and environmental sampling. Pigs originating from ABF and conventional farms were slaughtered at separate processing facilities. The ABF pigs were slaughtered at two small processing plants with an overall capacity of processing 250 pigs each per day. These small processing plants utilized an overnight chiller (1 to 4°C) to cool the carcasses and processed only ABF reared pigs. No commercial pigs were slaughtered at the small-scale processing facility. The conventionally raised pigs were processed in a large processing plant with a daily capacity of processing 9,000 pigs. The large slaughter plant utilized a blast chiller (−30°C for 2 h) to quickly cool the carcasses. At slaughter, mesenteric lymph nodes (MLN) and postevisceration and postchill swabs from ABF ($n = 565$) and conventionally raised ($n = 798$) pigs were collected. A total of 115 ABF and 90 conventional environmental samples were collected. These samples included swabs from trucks transporting the pigs to the processing plant (ABF, 35; conventional, 40) and lairage (resting area) swabs (ABF, 80; conventional, 50) after the animals arrival at the swine processing facility. All of the swabs collected at slaughter were premoistened with 10 ml of buffered peptone water. Swabs and MLN were placed in sterile Whirl-Pack bags upon collection. Postevisceration and postchill swabs were collected along the midline of the carcass from the jowl to the rear using sterile 10-by-10-cm^2 U.S. Department of Agriculture-approved plastic templates (International Bioproducts, Bothell, WA). After collection, the samples were immediately transported on ice for laboratory processing.

***Campylobacter* isolation and confirmation.** Upon arrival, a loopful of fecal samples were directly plated onto Campy-Cefex agar (27) and incubated under microaerophilic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen) in a water jacketed CO_2 incubator (Thermo Scientific, Inc., Waltham, MA) for 48 h at 42°C. Environmental samples were incubated in 30 ml of Bolton broth (Oxoid, Hampshire, United Kingdom) supplemented with laked horse blood (Hemostat Laboratories, Dixon, CA) and selective supplements (Oxoid, Hampshire, United Kingdom) at 42°C under microaerophilic conditions for 48 h before being plated onto Campy-Cefex and Mueller-Hinton agar. A single presumptive *Campylobacter* colony per sample was subcultured onto Mueller-Hinton agar (Becton Dickinson) and incubated using the temperature and conditions described above. Biochemical testing of presumptive colonies was performed through oxidase and catalase reactions (Becton Dickinson). A multiplex PCR protocol targeting both *C. coli* and *C. jejuni* (6) was used for the final confirmation of the *Campylobacter* isolates. Slight modifications to the amplification reaction included 0.5 μl of bovine serum albumin solution (20 mg/ml; Roche Diagnostics Corp., Indianapolis, IN), 0.25 μM concentrations of each primer, and 5 U of *Taq* DNA polymerase/μl.

Antimicrobial susceptibility testing. Confirmed *Campylobacter* isolates were tested for their susceptibility to a panel of nine antimicrobials by the broth microdilution method (Sensititre; Trek Diagnostics Systems, Ohio). The antimicrobials tested and the concentrations included: azithromycin (AZI; 0.015 to 64 μg/ml), ciprofloxacin (CIP; 0.015–64 μg/ml), erythromycin (ERY; 0.03 to 64 μg/ml), gentamicin (GEN; 0.12 to 32 μg/ml), tetracycline (TET; 0.06 to 64 μg/ml), florfenicol (FFN; 0.03 to 64 μg/ml), nalidixic acid (NAL; 4 to 64 μg/ml), telithromycin (TEL; 0.015 to 8 μg/ml), and clindamycin (CLI; 0.03 to 16 μg/ml). Briefly, 100 μl of cation adjusted Mueller-Hinton broth with TES buffer (Trek Diagnostics Systems) and bacterial inoculum adjusted to 0.5 McFarland were transferred to 11 ml of cation-adjusted Mueller-Hinton broth with TES buffer and lysed horse blood (Trek Diagnostics Systems). An automated delivery system (Sensititre AIM; Trek Diagnostics Systems) inoculated each plate with 100 μl of the previous culture. The plates were incubated for 24 h at 42°C under microaerophilic conditions. *C. jejuni* ATCC 33560 was used as the quality control organism. The MICs were recorded, and the values were compared to the breakpoints provided by the Clinical and Laboratory Standards Institute (7) as interpretive criteria. Isolates that were resistant to three or more antimicrobials were considered to be multidrug resistant (MDR).

Antimicrobial exposure in conventionally raised pigs. Antimicrobial use data at the conventional swine farms ($n = 30$) were collected from producers. The data were collected by production stage for sows (penicillin, 300,000 IU/ml) and piglets (penicillin, 150 IU/ml; enrofloxacin, 25 to 50 mg) at the farrowing, nursery (lincomycin, 32 g/gal; enrofloxacin, 50 mg; ceftiofur, 40 to 50 mg; penicillin, 300,000 IU/ml; carbadox, 50 g/ton; oxytetracycline, 400 g/ton; tiamulin, 35 g/ton; chlortetracycline, 400 g/ton; roxarsone, 27 g/ton), and finishing (chlortetracycline, 400 g/ton;

TABLE 1 *Campylobacter* prevalence on ABF and conventional farms

Sampling stage	Farm type[a]	*Campylobacter* prevalence (%) and significance in pig samples and environmental samples[b]													
		Pig feces		Water		Soil		Feed		Swabs		Lagoon		Farm truck	
		Prevalence	*P*	Prevalence	*P*	Prevalence	*P*	Prevalence	*P*	Prevalence	*P*	Prevalence	*P*	Prevalence	*P*
Farrowing[c]	ABF	41.3 (117/283)	0.20	12.5 (5/40)	0.62	7.7 (3/39)	0.75	2.5 (1/40)	1.00	25 (10/40)	0.13	NA	NA	NA	NA
	Conv	48.1 (202/420)		16 (8/50)		4 (2/50)		6 (3/50)		48 (24/50)		64 (32/50)		NA	
Nursery	ABF	83.7 (412/492)	0.06	39.5 (32/81)	0.74	17.5 (14/80)	0.35	0 (0/80)	0.22	33.8 (27/80)	0.42	NA	NA	NA	NA
	Conv	73.1 (476/651)		43 (43/100)		11 (11/100)		4 (4/100)		41 (41/100)		52 (52/100)		8.6 (3/35)	
Finishing	ABF	80.6 (374/464)	0.24	39 (30/77)	0.47	25 (20/80)	0.05	2.5 (2/80)	0.64	23.8 (19/80)	0.02	NA	NA	NA	NA
	Conv	72.7 (421/579)		52 (52/100)		3 (3/100)		15 (15/100)		44 (44/100)		55.8 (53/95)		13.3 (6/45)	

[a] ABF, antimicrobial-free; Conv, conventional.
[b] The number of positive *Campylobacter* samples/the total number of samples is indicated in parentheses. NA, not applicable. "Swabs" refers to drag swabs of floors and structures.
[c] The farrowing stage results include sows and piglets as follows: sows (ABF, 51.3%, 20/39; conventional, 71.4%, 50/70) ($P = 0.18$) and piglets (ABF, 39.8%, 97/244; conventional, 43.4%, 152/350) ($P = 0.65$).

tiamulin, 35 g/ton; penicillin, 300,000 IU/ml; virginiamycin, 10 g/ton) stages. Injectable antimicrobials, including penicillin, enrofloxacin, and ceftiofur, were administered for therapeutic purposes. Other antimicrobials, including carbadox, tiamulin, roxarsone, virginiamycin, chlortetracycline, and oxytetracycline, were administered as feed additives, except for lincomycin that was administered in the water for growth promotion.

Statistical analysis. Analysis of variance for repeated measures was performed with SigmaPlot 11.2 (Systat Software, Inc., Chicago, IL) to compare the *Campylobacter* prevalence and antimicrobial resistance profile from conventionally raised and ABF pigs. The prevalence and antimicrobial resistance of sows, carcasses, and environmental samples were compared by using a Student *t* test. A *P* value of <0.05 was considered statistically significant. Univariate analysis (SAS 9.1.3; SAS Institute Inc., Cary, NC) was used to determine associations between antimicrobial exposure in conventionally raised pigs in different production stages and the *Campylobacter* antimicrobial resistance profile. We analyzed the data for enrofloxacin, tiamulin, tetracyclines (chlortetracycline and oxytetracycline), and lincomycin use on farms and the corresponding resistance observed against these antimicrobials. The data for other antimicrobials used on the farms (penicillin, ceftiofur, carbadox, roxarsone, and virginiamycin) were not included in the analysis since these are not part of the antimicrobial susceptibility testing panel (CAMPY Sensititre plate; Trek Diagnostics Systems). In addition, no cross-resistance to the antimicrobials tested was observed. A single antimicrobial was analyzed per outcome of interest (for AR *Campylobacter*) using chi-square or Fisher exact test when applicable. Significance was determined at $P < 0.05$. The likelihood of antimicrobial resistance after antimicrobial exposure was determined based on the odds ratio (OR) and 95% confidence interval (CI). A farm effect was not adjusted in the associations between antimicrobial use and AR *Campylobacter* isolates due to the limitations in sample size to control for 30 farms.

RESULTS

***Campylobacter* prevalence on farms and in the environment.** *C. coli* ($n = 2{,}557$, 99.8%) was the predominant species identified in the study population followed by *C. jejuni* ($n = 4$, 0.2%) that were isolated from ABF sows ($n = 2$) and drinking water ($n = 2$). The conventionally raised sows had a higher prevalence (71.4%, 50/70) than ABF sows (ABF, 51.3%, 20/39) with no statistically significant difference between production systems ($P = 0.18$). There were no significant differences between the prevalences of ABF (72.9%, 903/1,239) and conventionally raised (66.6%, 1,099/1,650) pigs at the three sampling stages (farrowing, $P = 0.20$; nursery, $P = 0.06$; finishing, $P = 0.24$) (Table 1). Within individual production systems, we detected higher prevalence at nursery (ABF, 83.7%; conventional, 73.1%) and finishing (ABF, 80.6%; conventional, 72.7%) compared to the respective piglets at the farrowing stages. The overall *Campylobacter* prevalence in the environmental samples on conventional (29.9%, 396/1,325) farms was higher than on the ABF farms (20.5%, 163/797). *Campylobacter* was successfully recovered from water, soil, feed, drag swabs, lagoon, and truck samples (Table 1). The *C. coli* prevalence was higher in water (ABF, 12.5%, 5/40; conventional, 16%, 8/50; $P = 0.62$), swabs (ABF, 25%, 10/40; conventional, 48%, 24/50; $P = 0.13$), and lagoon (conventional, 64%, 32/50) samples at farrowing. At the nursery stage, a higher number of *C. coli* isolates were obtained from water (ABF, 39.5%, 32/81; conventional, 43%, 43/100; $P = 0.74$), swab (ABF, 33.8%, 27/80; conventional, 41%, 41/100; $P = 0.42$), lagoon (conventional, 52%, 52/100), and soil (ABF, 17.5%, 14/80; conventional, 11%, 11/100; $P = 0.35$) samples. The *C. coli* prevalence in the finishing environment was greater in water (ABF, 39%, 30/77; conventional, 52%, 52/100; $P = 0.47$), swabs (ABF, 23.8%, 19/80; conventional, 44%, 44/100; $P = 0.02$), soil (ABF: 25%, 20/80; conventional, 3%, 3/100; $P = 0.05$), lagoon (conventional, 55.8%, 53/95), feed (ABF, 2.5%, 2/80; conventional, 15%, 15/100; $P = 0.64$), and truck (conventional, 13.3%, 6/45) samples.

***Campylobacter* prevalence at slaughter and in the environment.** *Campylobacter* was isolated from MLN (ABF, 11.4%, 21/182; conventional, 28.8%, 78/271; $P < 0.01$), postevisceration (ABF, 73.1%, 133/182; conventional, 27.9%; 76/272; $P < 0.01$), and postchill swab (ABF, 1.0%, 2/199; conventional, 1.6%, 4/255; $P = 0.76$) samples (Table 2). Most of the isolates were speciated as *C. coli* ($n = 341$; 98.3%), whereas the *C. jejuni* isolates ($n = 6$; 1.7%) were obtained from the MLN. The *C. coli* prevalence of ABF postevisceration swabs (73.1%) was higher than the conventional samples (27.9%), as shown in Table 2 ($P < 0.01$). The MLN prevalence was significantly higher ($P < 0.01$) in the carcasses of conventionally raised pigs compared to the ABF prevalence. *C. coli* was also isolated from the slaughter environment (Table 2) of ABF (14.8%, 17/115) and conventional (17.8%, 16/90) samples, with the highest prevalence in lairage swabs (ABF, 21.3%, 17/80; conventional, 20%, 10/50; $P = 0.45$). *C. coli* were detected in trucks transporting conventionally raised pigs (15%, 6/40) but were not recovered from ABF truck samples.

***Campylobacter* AR profile on farms.** The AR profile of *C. coli* isolated from pigs and their environment at different sampling

TABLE 2 *Campylobacter* prevalence in ABF and conventional carcasses and in the environment at slaughter

Source	Sample type	Production type	*Campylobacter* prevalence (%)[a]	*P*
Carcass	Postevisceration	ABF	73.1 (133/182)	<0.01
		Conventional	27.9 (76/272)	
	MLN	ABF	11.4 (21/184)	<0.01
		Conventional	28.8 (78/271)	
	Postchill	ABF	1.0 (2/199)	0.76
		Conventional	1.6 (4/255)	
Environment	Lairage	ABF	21.3 (17/80)	0.45
		Conventional	20 (10/50)	
	Slaughter truck	ABF	0 (0/35)	0.02
		Conventional	15 (6/40)	

[a] The number of positive *Campylobacter* samples/the total number of samples is indicated in parentheses.

stages is presented in Table 3. Most of the *C. coli* isolates (76%, 1,944/2,557) were resistant to one or more antimicrobials, except for FFN. All of the *C. jejuni* farm isolates ($n = 4$) were pansusceptible. The frequency of resistance detected in the pigs at the farrowing, nursery and finishing stages on farms was highest to TET (ABF, 48.2%, 435/903; conventional, 88.3%, 970/1,099; $P < 0.01$), followed by AZI (ABF, 27.7%, 250/903; conventional, 25.3%, 278/1,099; $P = 0.73$) and ERY (ABF, 27.5%, 248/903; conventional, 25.2%, 277/1,099; $P = 0.72$). A similar level of resistance to TEL was observed in ABF (20.9%, 189/903) and conventionally raised (15.7%, 172/1099) pigs ($P = 0.81$). Isolates from conventionally raised and ABF pigs exhibited the same AZI, ERY, and TEL MICs (MIC_{50} and MIC_{90}). Conversely, for conventionally raised pigs the CIP and NAL MIC_{90}s were higher than for the ABF isolates (Table 3). MDR isolates were observed in both production systems at farm at all of the stages, including sows (ABF, 20%, 4/20; conventional, 10%, 5/50; $P = 0.63$) and piglets (ABF, 18.6%, 18/97; conventional, 27%, 41/152; $P = 0.50$) at the farrowing stage, the nursery stage (ABF, 21.4%, 88/412; conventional, 43.1%, 205/476; $P = 0.06$), and the finishing stage (ABF, 35.6%, 133/374; conventional, 42%, 177/421; $P = 0.25$). The most common MDR pattern in conventionally raised pigs throughout the three sampling stages was CIP-TET-NAL (14.3%, 157/1,099). Conversely, this pattern was only observed in a single ABF pig at the nursery stage. *C. coli* isolated from ABF pigs commonly exhibited the MDR pattern AZI-ERY-TET-TEL (9.5%; 86/903). Identical AR patterns found in both production systems included AZI-ERY-TET (ABF, 4.7%, 42/903; conventional, 8.2%, 90/1,099), AZI-ERY-TET-TEL (ABF, 9.5%, 86/903; conventional, 11.2%, 123/1,099), and AZI-ERY-TET-TEL-CLI (ABF, 8.2%, 74/903; conventional, 2.2%, 24/1,099). Three *C. coli* pig isolates from different ABF farms were resistant to seven of the nine antimicrobials tested, including AZI-CIP-ERY-TET-NAL-TEL-CLI ($n = 2$) and AZI-ERY-GEN-TET-NAL-TEL-CLI ($n = 1$).

The farm environmental isolates were also resistant to multiple antimicrobials except for GEN and FFN (Table 3). The frequency of resistance was highest to TET (ABF, 44.2%, 72/163; conventional, 86.4%, 342/396; $P < 0.01$). A statistically significant difference was observed for CIP resistance in the ABF and conventional farm environments ($P < 0.01$). The resistance pattern CIP-TET-NAL was commonly found in water (22/103), feed (1/22), swabs (21/109), soil (4/16), truck (2/9), and lagoon (23/137) isolates from conventional farms. In the ABF environment we observed eight different MDR patterns, among which AZI-ERY-TEL-CLI was predominant in water (8/67), swabs (5/56), and soil (3/37).

***Campylobacter* AR profile at slaughter.** A total of 341 *C. coli* and 6 *C. jejuni* isolates obtained from ABF ($n = 173$) and conventional ($n = 174$) pig carcasses and slaughter environments were tested. Similar to isolates from the farm, isolates from ABF and conventional pig carcasses had the highest frequency of resistance to TET (ABF, 49.4%, 77/156; conventional, 82.9%, 131/158; $P < 0.01$), followed by AZI (ABF, 34.6%, 54/156; conventional, 20.9%, 33/158; $P = 0.31$) and ERY (ABF, 34%, 53/156; conventional, 20.9%, 33/158; $P < 0.01$) (Table 3). *C. jejuni* isolates from MLN ($n = 6$) were susceptible to all of the antimicrobials tested except for a single TET-resistant isolate. *C. coli* isolates resistant to AZI, ERY, and TEL exhibited the same MIC_{90} in ABF and conventional pig carcass isolates, whereas the MIC_{90} differed for CIP and NAL. MDR *C. coli* isolates were isolated from MLN (ABF, 14.3%, 3/21; conventional, 29.5%, 23/78; $P = 0.24$), postevisceration (ABF, 33%, 44/133; conventional, 34.2%, 26/76; $P = 0.65$), and postchill (conventional, 25%, 1/4) samples. Common resistance patterns observed in the two production systems included AZI-ERY-TEL-CLI (ABF, 7/156; conventional, 1/158); AZI-ERY-TET (ABF, 3/156; conventional, 19/158) and AZI-ERY-TET-TEL (ABF, 27/156; conventional, 9/158). Postevisceration *C. coli* isolates from ABF (AZI-ERY-TEL, $n = 1$) and conventional (AZI-CIP-ERY-NAL, $n = 2$; AZI-CIP-ERY-NAL-TEL, $n = 1$) farms exhibited MDR patterns that were not observed at the farm level. Isolates from the slaughter environment of conventionally raised pigs also presented a higher frequency of resistance to TET (56.3%, 9/16). MDR *C. coli* isolates were observed in ABF (AZI-ERY-CLI, $n = 1$; AZI-ERY-TEL-CLI, $n = 1$; AZI-ERY-TET-TEL, $n = 2$; AZI-CIP-ERY-TET-NAL-TEL-CLI, $n = 1$) and conventionally raised (AZI-ERY-TET, $n = 1$; CIP-TET-NAL, $n = 1$) pig lairages. A single *C. coli* isolate from a truck was MDR (CIP-TET-NAL). Equal numbers of *C. coli* isolates ($n = 5$, 29.4%) from the ABF slaughter environment were resistant to AZI, ERY, and TET (Table 3). *C. coli* isolates resistant to the combination of AZI-ERY-TET-TEL were detected in conventional (1/16) and ABF (2/17) slaughter environments.

MDR profile comparison between farms, at slaughter, and in the environment. Similar MDR patterns were observed throughout the production chain on farms, at slaughter, and in the environment in both production systems. The predominant *C. coli* MDR phenotype (AZI-ERY-TET-TEL) observed in ABF pigs was also detected in the farm environmental samples. The same pattern was again detected in the ABF processing plant lairage area (2/17), in postevisceration carcass swabs (25/133), and in MLN (2/21). We found further evidence of the dissemination of an MDR *C. coli* exhibiting the AZI-ERY-TEL-CLI pattern in a single cohort of ABF pigs at all of the farm stages, including farrowing (5/117), nursery (13/412), and finishing (6/374); in the farm environment, including soil (3/37), swabs (5/56), and water (8/67); and again at the slaughter stages, including the MLN (1/21) and postevisceration (3/133) samples. In contrast to the findings presented above, the MDR patterns AZI-ERY-TET (52/1,239) and AZI-ERY-TET-TEL-CLI (86/1,239) were detected at the farm level in ABF pigs and their environment and at slaughter in postevisceration isolates but not in the slaughter plant environment.

The predominant *C. coli* MDR patterns in conventional farms

TABLE 3 *Campylobacter* antimicrobial resistance in ABF and conventional production systems

Antimicrobial and parameter[a]	Farm						Slaughter					
	Pig			Environment			Carcass			Environment		
	ABF (n = 903)	Conventional (n = 1,099)	P	ABF (n = 163)	Conventional (n = 396)	P	ABF (n = 156)	Conventional (n = 158)	P	ABF (n = 17)	Conventional (n = 16)	P
AZI												
MIC_{50}	0.12	0.12		0.12	0.12		0.12	0.06		0.12	0.06	
MIC_{90}	>64	>64		>64	>64		>64	>64		>64	32.06	
R (%)	27.7	25.3	0.73	34.4	15.4	0.01	34.6	20.9	0.31	29.4	12.5	0.34
CIP												
MIC_{50}	0.12	0.12		0.12	0.12		0.06	0.12		0.06	0.12	
MIC_{90}	0.25	8		0.25	16		0.12	8		0.12	2.06	
R (%)	1.2	17.1	0.11	0.6	20.5	<0.01	0	15.2	<0.01	5.9	12.5	0.75
ERY												
MIC_{50}	1	1		1	1		1	0.5		1	1	
MIC_{90}	>64	>64		>64	>64		>64	>64		>64	32.5	
R (%)	27.5	25.2	0.72	33.7	15.2	0.01	34.0	20.9	0.31	29.4	12.5	0.34
GEN												
MIC_{50}	0.5	0.5		1	1		0.5	0.5		0.5	0.5	
MIC_{90}	1	1		1	1		1	1		1	1	
R (%)	0.1	0	0.27	0	0		0	0		0	0	
TET												
MIC_{50}	8	>64		8	>64		8	>64		1	16	
MIC_{90}	>64	>64		>64	>64		64	>64		44.8	64	
R (%)	48.2	88.3	<0.01	44.2	86.4	<0.01	49.4	82.9	<0.01	29.4	56.3	0.38
FFN												
MIC_{50}	1	1		1	1		1	1		1	0.75	
MIC_{90}	1	1		1	1		1	1		1	1	
NS (%)	0	0		0	0		0	0		0	0	
NAL												
MIC_{50}	<4	8		<4	8		<4	<4		<4	<4	
MIC_{90}	8	>64		8	>64		8	64		11.2	36	
R (%)	1.3	16.7	0.11	0.6	19.9	<0.01	0	15.2	<0.01	5.9	12.5	0.75
TEL[b]												
MIC_{50}	2	2		2	2		2	1		1	1	
MIC_{90}	>8	>8		>8	>8		>8	>8		>8	5	
R (%)	20.9	15.7	0.81	19.6	10.6	0.57	26.3	7.0	0.18	23.5	6.3	0.22
CLI												
MIC_{50}	0.25	0.25		0.25	0.25		0.25	0.25		0.25	0.25	
MIC_{90}	8	4		8	2		6	2		8	1.125	
R (%)	12.5	3.5	0.04	20.2	3.0	<0.01	10.3	0.6	0.48	17.6	0	0.34

[a] Antimicrobials: AZI, azithromycin; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; FFN, florfenicol; NAL, nalidixic acid; TEL, telithromycin; CLI, clindamycin. Resistance breakpoints: AZI, ≥8 μg/ml; CIP, ≥4 μg/ml; ERY, ≥32 μg/ml; GEN, ≥8 μg/ml; TET, ≥16 μg/ml; NAL, ≥64 μg/ml; CLI, ≥8 μg/ml. R (%) = number of resistant isolates/no. of isolates tested; NS (%) refers to nonsusceptible (MIC ≥ 8 μg/ml), since the resistance breakpoint has not been determined. MICs are expressed as μg/ml.
[b] According to the CLSI, the telithromycin breakpoint is ≥16 μg/ml. The highest concentration on the CAMPY Sensititre plate was 8 μg/ml. Isolates with an MIC of ≥8 μg/ml were considered resistant.

were also found throughout the different sampling stages. Four farms with a predominant CIP-TET-NAL (250/1,495) MDR pattern were detected in pigs (piglets, 4/152; nursery, 88/476; finishing, 65/421) and farm environmental isolates (lagoon, 23/137; feed, 1/22; soil, 4/16; swabs, 21/109; water, 22/103). *C. coli* with the same pattern was also isolated from carcasses (MLN, 12/78; postevisceration, 6/76) and the slaughter plant environment (lairage, 1/10; truck, 1/6).

Associations between antimicrobial exposure and resistance in *C. coli*. The associations between antimicrobial use in conventionally raised pigs and the *C. coli* AR profile by production stage are presented in Table 4. The results at farrowing revealed a statistically significant association between the use of enrofloxacin and *C. coli* resistance to CIP and NAL ($P < 0.01$). However, the OR and CI values could not be estimated for this association due to unobserved resistance in pigs that did not receive enrofloxacin.

TABLE 4 Associations between antimicrobial exposure in pigs and resistance in *C. coli* by farm stage[a]

	Antimicrobial use on farms		Antimicrobial susceptibility testing					
Farm stage[b]	Antimicrobial	No. pigs exposed/total no. of pigs (%)	Antimicrobial tested[d]	No. of resistant pigs/no. of pigs exposed (%)	No. of resistant pigs/no. of pigs not exposed (%)	*P*[c]	OR	95% CI
Farrowing ($n = 202$)	ENR	60/202 (29.7)	CIP	5/60 (8.3)	0/142	<0.01*	NA	NA
			NAL	5/60 (8.3)	0/142	<0.01*	NA	NA
Nursery ($n = 476$)	LIN	45/476 (9.5)	CLI	1/45 (2.2)	15/431 (3.5)	0.65	0.6	0.1–4.9
	ENR	45/476 (9.5)	CIP	27/45 (60)	78/431 (18.1)	<0.01	6.8	3.6–12.9
			NAL	27/45 (60)	74/431 (17.2)	<0.01	7.2	3.8–13.8
	OTC	386/476 (81.1)	TET	348/386 (90.2)	88/90 (97.8)	0.01	0.2	0.05–0.9
	CTC	476/476 (100)	TET	436/476 (91.6)	NA	NA	NA	NA
	TIA	386/476 (81.1)	AZI	76/386 (19.7)	42/90 (46.7)	<0.01	0.3	0.2–0.5
			ERY	76/386 (19.7)	42/90 (46.7)	<0.01	0.3	0.2–0.5
Finishing ($n = 421$)	CTC	369/421 (87.6)	TET	341/369 (92.4)	49/52 (94.2)	0.63	0.7	0.2–2.5
	TIA	369/421 (87.6)	AZI	90/369 (24.4)	24/52 (46.2)	<0.01	0.4	0.2–0.7
			ERY	89/369 (24.1)	24/52 (46.2)	<0.01	0.4	0.2–0.7

[a] Abbreviations: ENR, enrofloxacin; LIN, lincomycin; TIA, tiamulin; OTC, oxytetracycline; CTC, chlortetracycline; CIP, ciprofloxacin; NAL, nalidixic acid; CLI, clindamycin; TET, tetracycline; AZI, azithromycin; ERY, erythromycin. NA, not applicable.
[b] n = the total number of pigs at the production stage.
[c] Determined using the chi-square except as noted. *, Determined using the Fisher exact test.
[d] These antimicrobials are included in the *Campylobacter* susceptibility testing panel.

Even though tiamulin, tetracyclines, and lincomycin were not administered to farrowing pigs, *C. coli* isolates resistant to AZI (22.8%, 46/202), ERY (22.8%, 46/202), TET (71.3%, 144/202), and CLI (5%, 10/202) were observed. At the nursery stage, significant associations were determined between exposure to enrofloxacin and resistance to CIP ($P < 0.01$) and NAL ($P < 0.01$), between oxytetracycline (OTC) exposure and TET resistance ($P = 0.01$), and between tiamulin exposure and *C. coli* resistance to AZI ($P < 0.01$) and ERY ($P < 0.01$). Pigs that received enrofloxacin at nursery were 6.8 (95% CI = 3.6 to 12.9) and 7.2 (95% CI = 3.8 to 13.8) times more likely to develop resistance to CIP and NAL, respectively. After exposure to OTC, *C. coli* from nursery pigs were 0.2 (95% CI = 0.05 to 0.9) times as likely to be resistant to TET. Resistance to TET was also observed in a high percentage of *C. coli* isolates from pigs not exposed to OTC (97.8%, 88/90). Pigs that received tiamulin were 0.3 (95% CI = 0.2 to 0.5) times as likely to develop resistance to AZI and ERY. There was no statistically significant association between lincomycin use and CLI resistance in nursery pigs ($P = 0.65$). A chi-square test showed a significant association between tiamulin exposure at the finishing stage and AZI ($P < 0.01$) and ERY ($P < 0.01$) resistance. Resistance to CIP (18.5%, 78/421), NAL (18.3%, 77/421), and CLI (3.1%, 13/421) was detected in *C. coli* isolates from pigs at the finishing stage even though enrofloxacin and lincomycin were not administered.

DISCUSSION

The objective of this longitudinal study was to determine the prevalence and antimicrobial resistance profile of *Campylobacter* isolated from pigs reared in ABF and conventional production systems on farms and at slaughter. *C. coli* was the predominant species in swine from ABF and conventional farms in accordance with the previous findings (22, 31). *C. coli* was able to persist in pigs and in the environment in both production systems with different swine-rearing practices. The *C. coli* prevalence of sows and piglets at farrowing (ABF, 41.3%; conventional, 48.1%) suggests an early colonization of piglets (3, 38) and potential transmission from sows. It is also important to highlight that the prevalence in piglets could potentially indicate the role of the environment in pathogen transmission. We observed an increase in *C. coli* prevalence through the nursery and finishing stages in both ABF and conventionally raised pigs. Only a few studies have examined the status of *Campylobacter* in outdoor and indoor swine farm environments (3, 17). In our study, we isolated *Campylobacter* from water, swabs, soil, and feed on conventional and ABF farms, in addition to lagoon and truck samples in conventional farms only. Furthermore, a higher environmental prevalence was observed on the conventional farms. These observations demonstrate potential environmental sources in *Campylobacter* transmission even when AIAO practices are followed.

The persistence of MDR *C. coli* in all of the farm and slaughter stages in our study clearly indicates the ability of *C. coli* strains to disseminate across the production chain and persist in the farm and slaughter environments. To our knowledge, only a few reports have addressed the presence of *Campylobacter* in lymphoid organs. Studies of poultry have recovered *C. jejuni* from the bursa, thymus, and spleen (8, 9), while *Campylobacter* has been isolated from the palatine tonsils of fattening pigs at slaughter (13). In our study, the detection of *Campylobacter* in the MLN from ABF and conventional pig carcasses clearly indicates the ability of this pathogen to enter the lymphatic system from the gut. This information may be valuable since the pathogenesis of this bacterium is not fully understood. Moreover, the presence of *Campylobacter* in the MLN represents a potential for carcass contamination during processing procedures.

We detected *Campylobacter* in ABF and conventional postchill samples. Despite the fact that ABF and conventional processing plants differ in their carcass-chilling methods (i.e., overnight chilling versus blast chilling), we observed no major differences in *Campylobacter* survival from 2 to −30°C ($P = 0.76$). A significant difference between overnight chilling versus blast chilling ($P <$

0.001) was observed in previous findings from our laboratory (31). In line with the previous study, both chilling methods successfully decreased the *Campylobacter* prevalence at postchill, which is a reflection of the effect freezing temperatures have on its survival (5). Regardless of this significant decrease, the presence of *C. coli* at the closest stage to the final product represents food safety concerns. The slaughter environment serving as a source of *Campylobacter* was demonstrated in results from the lairage and slaughter truck floor samplings. Detection of *C. coli* in trucks used to transport conventionally raised pigs before the animals were transported to the processing facility and again in the lairage of ABF and conventional processing plants indicates the possibility of pathogen transmission to pigs from environmental sources. Likely reasons for the absence of *Campylobacter* in the ABF slaughter trucks include a smaller truck size and reduced pig loads, facilitating cleaning and disinfection practices.

Antimicrobials are used in the conventional swine industry for disease treatment and to enhance the growth of animals (10, 36). Antimicrobial resistance in conventional farms has been previously documented (22, 31). Similar to previous reports (2, 20), growth promoters, including tetracyclines and tiamulin, were administered in the feed of growing pigs. Therefore, a higher frequency of resistance to tetracycline was not surprising in *C. coli* from conventionally raised pigs. *C. coli* resistance to macrolides (AZI and ERY) could be the result of cross-resistance to the use of tiamulin, as has been demonstrated in *Mycoplasma gallisepticum* (18). Even though ciprofloxacin is not used in swine production, cross-resistance could develop through the use of enrofloxacin (11). Resistance to ciprofloxacin is mostly determined by chromosomal mutations; however, studies have demonstrated the horizontal transfer of resistance genes through plasmids (28), which could further facilitate the emergence of ciprofloxacin-resistant *Campylobacter*. Cross-resistance has also been suggested between lincomycin and clindamycin since they belong to the same antimicrobial class (35). The MDR pattern CIP-TET-NAL was predominant among other patterns in conventionally raised pigs, in contrast to findings by other authors (22, 31). Antimicrobial resistance to ciprofloxacin and macrolides is a concern due to their importance in treating humans.

The association of antimicrobial exposure and the development of resistance in pigs has been previously addressed (2, 23). In the present study, significant associations were determined between exposure to enrofloxacin, tetracycline, and tiamulin and resistance development in *C. coli* obtained from conventionally raised pigs. *C. coli* resistance to these antimicrobials was 0.2 to 7.2 times more likely to develop after antimicrobial use. The high prevalence of MDR *C. coli* isolates from pigs reared in the ABF system, which did not receive antimicrobials for treatment or growth promotion, clearly indicates that the absence of antimicrobial use does not necessarily result in the absence of AR bacterial populations. In addition, similar MIC_{50} and MIC_{90} values were observed for majority of the antimicrobials in *Campylobacter* isolates from ABF and conventionally raised pigs. Different MDR patterns were observed in ABF pigs from farrowing to finishing, including unique patterns with resistance to seven antimicrobials. These ABF MDR patterns also included resistance to telithromycin, which has not been previously reported in ABF pigs. The results of our study provide evidence of AR *C. coli* strains in the environments of ABF and conventional farms that correlated with patterns observed in the animals themselves. For example, the MDR pattern AZI-ERY-TET-TEL was observed in pigs from farrowing to finishing and in farm environmental isolates from water, feed, swabs, and soil. Furthermore, for some antimicrobials (AZI, ERY, and CLI) the resistance observed was significantly higher in the ABF farm environment. In all cases, the environmental *C. coli* strains had the same MDR patterns as those detected in the pigs on farms and at slaughter.

Observations in pigs and in farm environment prove to be true also at slaughter, with more MDR phenotypes in ABF *C. coli* strains at postevisceration. Previous studies have also detected MDR *C. coli* at slaughter (15, 31), observing up to four MDR patterns. In our study most of the MDR profiles at slaughter were also observed at the farm level for both production systems. The few exceptions suggest acquisition due to cross-contamination during processing since these resistance phenotypes were exclusive to postevisceration isolates. Despite the *C. coli* prevalence observed in the conventional slaughter trucks, it appears that these do not play an important role in the transmission of MDR *C. coli* since only a single isolate was MDR. Interestingly, more MDR isolates were present in the ABF lairage, including the strain exhibiting resistance to seven antimicrobials detected at the ABF farm. The AR results observed at slaughter further highlight the potential role played by the environment in the transmission of MDR *C. coli* to pigs on farms and at slaughter.

Even though it is not possible to determine the direction of pathogen transmission between pigs and the environment, we can clearly see that both pig and environmental isolates share common phenotypic characteristics, as illustrated by their resistance profiles. These observations reflect the possible exchange of MDR *C. coli* populations between pigs and the environment, affecting the phenotypic diversity of *Campylobacter* on farms and at slaughter. It remains unclear how MDR *C. coli* is able to adapt and persist in the environment, particularly considering the fitness cost this may represent to strains that survive under no antimicrobial selection pressure (4).

In summary, we determined that *C. coli* is prevalent in both ABF and conventional production systems on farms, at slaughter, and in the environment. MDR isolates were found throughout all of the production stages and environment even in the absence of antimicrobial selection pressure. Identical resistance profiles were observed between the pigs and their environment on farms and at slaughter. Our results highlight the role of the environment in the persistence and dissemination of AR *Campylobacter*, particularly in alternative swine production systems that do not use antimicrobials. The environmental reservoirs present in ABF and conventional farms could potentially explain resistance in pigs that were not exposed to antimicrobials. The prevalence of AR bacterial strains in food animals raised in the ABF production systems where no antimicrobials are used for either treatment or prophylaxis is a cause for concern. The information we provide here should be considered in efforts to reduce the public health threat of AR *Campylobacter*.

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