Antimicrobial resistance in *Campylobacter* isolated from food animals and humans in northern Thailand

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Abstract

A study was conducted to determine the prevalence of *Campylobacter* with antimicrobial resistance from chickens, pigs, dairy cows, healthy farm workers, and children hospitalized with diarrhea in northern Thailand. Resistance was highest in pig samples and lowest in healthy farm workers. Resistance to fluoroquinolones and tetracycline was high in all study populations. The increased prevalence of resistant isolates from meat samples collected at markets, compared to isolates collected from animals on the farm or the slaughterhouse, suggests that contamination after carcasses leave the slaughterhouse is an important factor in the spread of resistant bacteria into the human food chain.

Introduction

Campylobacter ssp. is a major foodborne bacterium affecting mainly children in developing countries and young adults in industrialized countries (Oberhelman & Taylor, 2000; Coker *et al*., 2002). The clinical signs of *Campylobacter* infection in humans include fever, myalgia, and arthralgia (Sanders *et al*., 2002), and it has been reported as a cause of travelers' diarrhea (Hakkanen *et al*., 2003). In Thailand, *Campylobacter* was the most common pathogen found in children less than 12 years old with dysentery (Bodhidatta *et al*., 2002).

The main source of *Campylobacter* in human infections is believed to be from consumption of foods of animal origin (Osano & Arimi, 1999; Kalman *et al*., 2000) or other cross-contaminated foods, and from drinking contaminated water (Kapperud *et al*., 2003). Previous studies in Thailand showed the prevalence of *Campylobacter* ssp. to be over 60% in broilers (Padungtod *et al*., 2002) and 12% in foods of animal origin purchased at market (Rasrinual *et al*., 1988). Fortunately, only a few species of *Campylobacter* result in clinical disease in animals, including *Campylobacter fetus* ssp. *fetus* and *Campylobacter fetus* ssp. *venerealis,* which cause reproductive diseases (Sanders *et al*., 2002). Other species of *Campylobacter*, including those causing enteritis in humans, do not result in clinical disease in animals. For this reason, infection in food animals can go unnoticed, and can be an important source of human infection and disease caused by *Campylobacter*.

Another issue of concern regarding *Campylobacter* is the increase in antimicrobial resistance appearing in various regions around the world (Bywater *et al*., 2004; Gupta *et al*., 2004; Hart *et al*., 2004). Infection to these resistant *Campylobacter* may lead to suboptimal outcomes of antimicrobial treatment (Sanders *et al*., 2002) or treatment failure (Butt *et al*., 2003). Antimicrobial resistance in both human and animal *Campylobacter* isolates has become increasingly common in Thailand and other developing countries (Isenbarger *et al*., 2002). An earlier study in Thailand found high proportions of *Campylobacter* resistant to a variety of antimicrobial agents, including fluoroquinolones (nalidixic acid and ciprofloxacin) (Padungtod *et al*., 2003).

Since the 1990s there has been evidence suggesting that the increased prevalence of *Campylobacter* with resistance to antimicrobial agents, particularly fluoroquinolones, may be a result of the use of these agents in food animals (Endtz *et al*., 1991; Smith *et al*., 1999), and that antimicrobial use in food animal production may contribute to the increase of antimicrobial-resistant bacteria in humans (Smith *et al*., 2002). However, in Thailand, it has been suggested that food animals such as poultry may only have a limited role in the

increased antimicrobial resistance (Boonmar *et al.*, 2005). Given these conflicting findings, this study was conducted to determine the prevalence and serotypes of *Campylobacter* with antimicrobial resistance, from chickens, pigs, dairy cows, healthy farm workers, and diarrhea patients in a farming community in northern Thailand. Comparison of antimicrobial susceptibility profiles of *Campylobacter* isolated from these populations may provide an insight into the development and transmission of antimicrobial-resistant *Campylobacter* in Thailand.

Materials and methods

Sampling sites

All sampling sites were located in the Chiang Mai and Lamphung provinces of northern Thailand, and were selected to be within 3 h (90 km) of the laboratory. These study locations included farms, slaughterhouses, fresh meat markets, and hospitals. In addition, the farms, slaughterhouses and markets selected needed to keep records so that animals could be tracked from the farm to the slaughterhouse, and subsequently from slaughter to the markets. Samples were collected and processed during May–July of 2000–2003. The prevalence of *Campylobacter* collected from chickens and pigs in this study was highest at the farm, and *Campylobacter* was isolated from 18% of children with diarrhea at the hospital (Padungtod & Kaneene, 2005).

The chicken and pig farms in this study were not large, industrialized facilities, but were parts of integrated production systems. Antimicrobial use on these farms was regulated by the companies purchasing these animals for slaughter. The only hygenic measures practiced were the use of footbaths at the entrances of animal housing units. Chicken samples were collected from broiler production farms, where large numbers of birds were kept in closed buildings. The chickens were fed coccidiostats (e.g. amprolium) and antimicrobial agents (including sulfamethoxazole and enrofloxacin) during the raising period, but were not treated with antimicrobial agents prior to sample collection. Pig samples were collected from finishing operations, with no sows or piglets housed on the premises. The pigs were housed in open buildings which did not exclude free-flying wild birds. Pigs in this study received lincomycin and tylosin prior to their arrival at the finishing operation, but no antimicrobial agents were present in feeds while at finishing. Dairy cattle in this study were adult animals from small farms (10–20 milking animals), housed in free-stall barns with little pasture access. High levels of hygiene were practiced on dairy farms, where milk prices depend on the cleanliness of the operation.

Individual animals were randomly selected for sampling, based on their age and stage of production, and were animals that did not receive antimicrobial treatment at the farm prior to sampling. Fecal samples were collected with swabs of the rectum (pigs, dairy cows) or cloaca (chickens). Pigs <1 month old prior to slaughter, chickens <2 weeks old prior to slaughter, and milking cows were included in the on-farm study. The same pigs and chickens were tracked and sampled at the slaughterhouse and again at the markets.

All workers on the farms participating in the study, and the parents of all children with diarrhea in the hospitals within the study area were contacted for participation while the child was hospitalized. Participants were excluded if they had received any antimicrobial treatments during the study period prior to sample collection. Due to the low numbers of possible human samples, efforts were made to contact and enroll all possible candidates. Approval to conduct research involving human subjects and animals was given by the Chiang Mai University Committees on Human Subjects and Animal Research, respectively. Farm workers usually lived on the farms where they worked, but did not live in animal housing units. The water supply to workers and animal may have come from the same sources. The pediatric population with diarrhea may have come from urban areas, where the water was treated with chlorine.

The slaughterhouses participating in our study were small-scale facilities providing meat for local market consumption, and efforts were made to sample animals from the farms participating in the study. The poultry slaughterhouses processed 500–800 birds per night. At the slaughterhouse, birds from various farms were kept together in the holding pen while waiting to be slaughtered by hand. After mechanical defeathering, carcasses were chilled in cold water without evisceration, since evisceration is done at the market to provide consumers with viscera for separate purchase. Slaughterhouse samples were collected from chickens from study farms, after defeathering and before chilling. The pig slaughterhouse in this study did not use machinery for the slaughtering process. After slaughter, the pigs were dehaired, eviscerated, and cut into six pieces by hand. The slaughterhouse samples were collected from the pigs from study farms at the end of the butchering process, but before shipment to market. At the slaughterhouse, samples were collected by swabbing an approximately 50 cm^2 area of the pig carcass with sterile gauze, and cotton swabs were used to swab under the wing and surrounding the cloaca of the chicken carcass. Samples of mesenteric lymph nodes were collected from pigs at the end of butchering. Pig carcasses, including visceral organs, were delivered to the market directly after slaughtering.

At the fresh meat market, approximately 100 g of pork from the neck area attached to the head (with the ear tag) and 200 g of thigh meat from each chicken were purchased. Efforts were made to sample animals from the farms participating in the study. All farm, slaughterhouse, and

market environmental samples were collected using a sterile gauze swab soaked in sterile skim milk, which was used as a transport media for these samples. All samples were held in an icebox or refrigerator until further processing, which was completed within 48 h of collection.

Bacterial isolation

All swabs and meat samples were suspended in 10 mL Bolton broth (BB; Oxoid, UK) supplemented with antimicrobial agents (cefoperazone, vancomycin, trimethoprim, amphotericin B) as enrichment media to resuscitate damaged cells and to limit the growth of other bacteria. The broth was incubated at 42 °C, 5% CO_2 for 48 h. A swab of the broth was inoculated onto Karmali (KSA) or Preston agar (PA; Oxoid) supplemented with antimicrobial agents (sodium pyruvate, vancomycin, cefoperazone, amphotericin B for KSA; polymyxin B, trimethoprim, rifampicin, cycloheximide for PA) as selective media. For fecal, cloacal, and rectal swabs, samples were inoculated directly on KSA. Selective media plates were incubated at 42 °C, 5% CO_2 for up to 5 days. A single colony of bacteria with *Campylobacter* characteristics was selected from each plate for biochemical tests. Gram-negative, spiral rods with positive oxidase and catalase tests were identified as *Campylobacter*, and grown on *Brucella* agar supplemented with sheep's blood (BASB). After 48 h of incubation at 42 °C, 5% CO_2, the bacteria were suspended in Mueller–Hinton broth, then mixed with an equal volume of 60% glycerol and stored in a −70 °C freezer.

Antimicrobial susceptibility testing

Our study used both the broth microdilution technique and disk diffusion technique to categorize *Campylobacter* into resistant and nonresistant groups. Both techniques were shown to yield comparable minimal inhibitory capacity (MIC) values and similar resistance classification results (Frediani-Wolf & Stephan, 2003).

For *Campylobacter* isolates collected from 2000–2002, antimicrobial susceptibility testing was done using the microbroth dilution technique following the National Committee on Clinical Laboratory Standards (NCCLS, 2000). The antimicrobial agents tested included ampicillin, azithromycin, chloramphenicol, gentamicin, nalidixic acid, tetracycline, ciprofloxacin, and erythromycin. Approximately 10^5 CFU per mL of bacterial suspension, turbidity adjusted to a 0.5 McFarland standard, was inoculated into a 96-well microtiter plate containing twofold dilutions of antimicrobial agents. After incubation in micro-aerobic conditions for 44–48 h, the MIC was determined by observing the growth of bacteria in each well. The MIC is the minimum concentration of an antimicrobial agent that inhibits growth of the bacteria. The breakpoints provided by the U.S. National Antimicrobial Resistance Monitoring System (NARMS, 2003) were used to categorize *Campylobacter* into resistant and nonresistant groups. *Staphylococcus aureus* NTCC25922 and *Escherichia coli* NTCC29213 were used as quality control organisms. Plate counts were conducted to confirm the concentrations of bacterial inoculum used in testing.

For *Campylobacter* isolates collected in 2003, reductions in funding necessitated the use of the less-expensive disk diffusion technique for antimicrobial susceptibility testing (Gaudreau & Gilbert, 1997). Approximately 10^8 CFU per mL bacterial suspensions were inoculated onto Mueller–Hinton agar. Commercially prepared antimicrobial disks (Oxoid) were used, and included ampicillin, chloramphenicol nalidixic acid, tetracycline, ciprofloxacin and erythromycin. After incubation in micro-aerobic conditions for 44–48 h, the zones of inhibition (in mm) were measured with calipers, and NCCLS breakpoints were used to categorize *Campylobacter* into resistant and nonresistant groups.

Speciation of *Campylobacter*

For isolates collected and isolated from 2000 to 2002, API-Campy (bioMérieux, Marcy l'Etoile, France) kits were used for species identification following the manufacturer's recommendations. For *Campylobacter* isolates collected in 2003, reductions in funding necessitated the use of a multiplex PCR assay (Wang *et al.*, 2002) to identify the species of *Campylobacter* isolate. In brief, the multiplex PCR was started by suspending a 48 h culture of the bacteria in sterile water. A phenol–chloroform extraction protocol (Ausubel *et al.*, 1999) was used to extract the DNA from the bacteria. Then, 2.5 μL of DNA template was added to the multiplex PCR mixture containing 1.25 unit of Taq DNA polymerase (Pacific Sciences, Bangkok, Thailand) reaction buffer (50 mM Tris-HCL, pH 8.3, 10 mM KCl, 5 mM $[NH_4]_2SO_4$), 20 mM $MgCl_2$, 200 μM deoxynucleoside triphosphate (dNTPs), and the 12 primers set. The multiplex PCR was carried out in a thermal cycler (Thermohybaid, Waltham, MA) using the following procedure: initial denaturation at 95 °C for 6 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 7 min. The resulting product was visualized by 1.5% agarose gel electrophoresis stained with $EtBr_2$. The species were identified based on the size of the product compared with a known *Campylobacter* ssp. control.

Statistical analysis

The prevalence of resistance was calculated by dividing the number of samples with resistant *Campylobacter* by the total number of *Campylobacter* processed. Testing for significant differences in the prevalence of resistant *Campylobacter* from various populations, locations, and sample types were

conducted using the Chi-squared test, or Fisher's exact test when numbers did not meet the necessary assumptions for the chi-squared test (cell counts < 5).

Results

The recovery rate of isolates for susceptibility testing from frozen isolate stocks was approximately 60%. A total of 686 *Campylobacter* isolates were tested, including 324 isolates from chickens, 319 from pigs, 15 from dairy animals, and 28 isolates from humans (Table 1). The remaining 24 were positive for the *Campylobacter* 23s RNA gene, but did not yield specific bands for species identification. Consequently, we were unable to speciate some isolates from chickens (14 on the farm, nine at slaughter, one at market), dairy cattle (6), pigs (1 on the farm), and children hospitalized with diarrhea (5). By species, *Campylobacter coli* made up the majority of recovered pig (50/51) and chicken (124/241) *Campylobacter* isolates, while *C. jejuni* was recovered from most dairy cattle (10/17) and some chicken (93/241) frozen isolates. The majority of *Campylobacter* recovered from healthy farm worker isolates were *C. coli* (3/5), whereas *C. jejuni* was the most prominent species recovered from isolates collected from children hospitalized with diarrhea (20/29). By host species on the farm, the highest levels of resistance seen were to nalidixic acid in isolates from chickens (60%), tetracycline in isolates from pigs (88%), and ciprofloxacin in dairy cattle isolates (29%). At slaughter, 90% of chicken isolates and 77% of pig isolates demonstrated resistance to nalidixic acid. Both chicken and pig isolates from the market demonstrated high levels of resistance to ciprofloxacin (91% and 100%, respectively). The most common form of resistance seen in *Campylobacter* isolates from healthy adults was to nalidixic acid (60%), whereas the majority of isolates from children hospitalized with diarrhea demonstrated resistance to erythromycin (78%).

There were significant differences ($P < 0.01$) in the prevalence of resistant *Campylobacter* among animals at the farm for all agents tested except ampicillin (Table 2). There were also significant differences in the prevalence of resistance between sampling locations for all agents tested except chloramphenicol and gentamicin.

In chickens, the highest prevalence of resistance to most agents was observed at the market, with the exception of azithromycin. The highest resistance was found to fluoroquinolones (ciprofloxacin and nalidixic acid) and tetracycline at all sampling points. Significant differences were observed for ciprofloxacin, nalidixic acid and tetracycline,

Table 1. Numbers of *Campylobacter* isolates tested for antimicrobial susceptibility

Host	Location	2000	2001	2002	2003	Total
Chickens	Farm	77	124	11		212
	Market		32			32
	Slaughterhouse	26	54			80
Pigs	Farm	43	152			195
	Market		13			13
	Slaughterhouse	38	73			111
Dairy Cattle	Farm			15		15
Humans	Farm	2		3		5
	Hospital				23	23
Total		186	448	29	23	686

Table 2. Proportion of *Campylobacter* isolates demonstrating antimicrobial resistance, by host and location

			Antimicrobial agents							
Host	Sample location	*n*	AMP	CIP	ERY	TET	NAL	CHL	GEN	AZI
Chicken*	Farm	212	0.0	54.2	5.8	52.8	60.3	0.0	0.0	5.8
	Slaughterhouse	80	nt	63.8	1.9	37.5	90.0	0.0	0.0	1.9
	Market	32	nt	90.6	3.1	81.3	81.3	0.0	0.0	3.1
Pig†	Farm	195	nt	78.0	83.4	87.5	84.5	1.3	12.1	82.8
	Slaughterhouse	111	nt	57.7	50.7	76.6	76.6	2.7	8.2	50.7
	Market	13	nt	100.0	46.2	92.3	92.3	0.0	0.0	46.2
Dairy cattle‡	Farm	15	17.7	29.4	5.9	11.8	11.8	5.9	0.0	11.8
Humans	Healthy§	5	33.3	20.0	33.3	40.0	60.0	0.0	0.0	33.3
	Hospitalized¶	23	30.4	69.6	78.3	34.8	65.2	nt	nt	nt
All		686	12.1	64.7	38.3	66.2	74.1	1.0	5.1	38.3

AMP, ampicillin; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline; NAL, nalidixic acid; CHL, chloramphenicol; GEN, gentamicin; AZI, azithromycin; nt, no isolates tested.

*51.5% *Campylobacter coli*.

†98% *Campylobacter coli*.

‡59% *Campylobacter jejuni*.

§60% *Campylobacter coli*.

¶69% *Campylobacter jejuni*.

but not for erythromycin and azithromycin. No resistance to chloramphenicol and gentamicin was observed in chickens.

In pigs, the highest prevalences of resistance were observed at market, except for the macrolides erythromycin and azithromycin, where resistance was highest on the farms. Significant differences were observed for all other agents except nalidixic acid, chloramphenicol, and gentamycin. In dairy cattle, resistance to ciprofloxacin was higher than other agents tested. In healthy farm workers, the prevalence of resistance was highest to nalidixic acid. In the diarrhea patients the prevalence of resistance was highest to erythromycin. No resistance to chloramphenicol and gentamicin was observed in humans.

There were significant ($P < 0.01$) differences in the numbers of antimicrobial agents to which *Campylobacter* isolates were resistant, based on the source population of the *Campylobacter* isolates (Fig. 1), but no significant difference was observed between sampling locations ($P = 0.07$). Multidrug resistance was found in *Campylobacter* isolates from all species tested. Chicken isolates were most frequently resistant to three or fewer agents, and isolates from dairy cattle and humans were most frequently resistant to one and two agents, respectively. The majority of *Campylobacter* isolates from pigs were resistant to five or fewer isolates, with the largest number of those isolates resistant to five antimicrobial agents. More multiresistant isolates were found in farm samples than slaughter samples for pigs and chickens: 89% of pig farm isolates and 66% of pig slaguther isolates were resistant to three or more agents, compared to 10% of chicken farm isolates and 4% of chicken slaughter isolates. Multi-resistance was low in chicken samples (3% resistant to three or more agents), but higher in pigs (57%). The most common combinations of multiresistance seen were to ciprofloxacin–nalidixic acid–tetracycline–erythromycin–azithromycin (143 isolates), and ciprofloxacin–nalidixic acid–tetracycline (135 isolates) (Table 3).

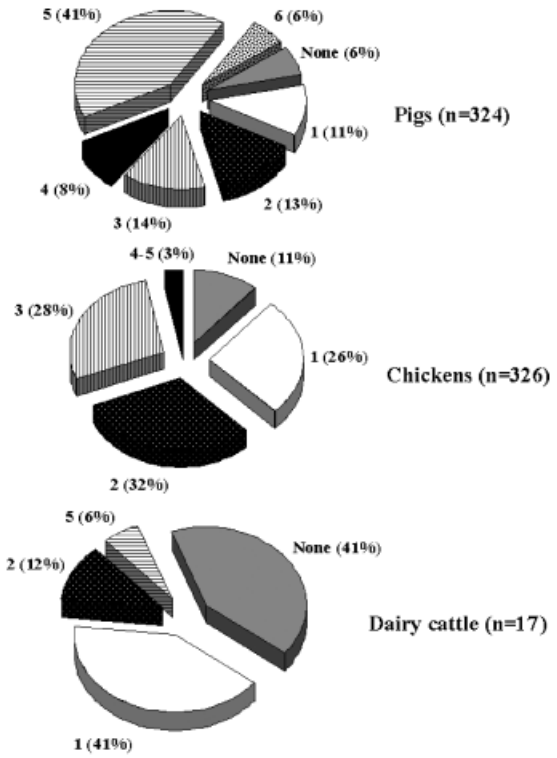

Fig. 1. Proportion of *Campylobacter* isolates demonstrating multidrug resistance to different numbers of agents (and percent of isolates), by source species.

Antimicrobial resistance patterns of *C. jejuni* and *C. coli* isolated from chicken fecal samples were compared to determine whether patterns of resistance differed based on the species of *Campylobacter* (Table 4). Resistance to

Table 3. Counts of *Campylobacter* ssp. with different resistance profiles ($n = 672$ isolates)

Profile	No. of isolates
No resistance demonstrated	66
Resistance to one agent	
CIP/NA*	72
NA only	59
TET	43
CIP only	24
CHL	2
AMP	1
Total	201
Resistance to two agents	
CIP/NA-TET	135
NA-TET	58
CIP-TET	19
NA-AZI	1
Total	213
Resistance to three agents	
CIP/NA-TET-GEN	6
CIP/NA-ERY-GEN	1
CIP/NA-TET-ERY	1
ERY-AZI-AMP	1
ERY-AZI-TET	1
Total	10
Resistance to four agents	
CIP/NA-ERY-TET-AZI	143
CIP-TET-ERY-AZI	16
NA-TET-ERY-AZI	2
Total	161
Resistance to five agents	
CIP/NA-ERY-TET-AZI-GEN	18
CIP/NA-ERY-TET-AZI-CHL	2
CIP-ERY-AZI-AMP-CHL	1
Total	21

*Combined resistance to ciprofloxacin/nalidixic acid in an isolate does not count as multi-drug resistance.

AMP, ampicillin; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline; NAL, nalidixic acid; CHL, chloramphenicol; GEN, gentamicin; AZI, azithromycin.

ciprofloxacin and nalidixic acid was significantly higher ($P \leq 0.05$) in *C. coli* than in *C. jejuni*, whereas tetracycline resistance was significantly higher in *C. jejuni* than *C. coli*. Higher levels of resistance to azithromycin, erythromycin, and clindamycin were found in *C. coli*, but these differences were not statistically significant. There were no significant differences between *Campylobacter* species in levels of multi-resistant isolates. Levels of resistance in isolates from fecal samples showed similar patterns of resistance to isolates from meat samples collected at the market, but the small number of *C. jejuni* isolated from market samples made tests for statistical significance impossible.

Comparisons of patterns of resistance between hosts were conducted with *C. coli* isolates, which were present in sufficient numbers in both host species to allow statistical analysis (Table 5). Isolates from pigs had significantly higher levels of resistance to azithromycin, clindamycin, erythromycin, gentamicin, and tetracycline than those from chickens. There were also higher levels of multi-resistant isolates of *C. coli* from pigs compared to isolates collected from chickens.

Table 4. Comparison of patterns of antimicrobial resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from chicken fecal samples collected at the farm, 2000–2001

	Campylobacter jejuni ($n = 56$)	*Campylobacter coli* ($n = 92$)	Fisher's Exact *P*
Ciprofloxacin	71.4	87.0	0.0293
Nalidixic acid	69.6	89.1	0.0041
Erythromycin	1.8	8.7	0.1538
Gentamicin	0	0	–
Azithromycin	1.8	9.8	0.0900
Chloramphenicol	0	0	–
Clindamycin	0	8.7	0.246
Tetracycline	66.1	51.1	0.0001
Multiresistant	51.8	50.0	0.8666

Discussion

Resistance to antimicrobial agents used in human therapy is increasing in pathogenic *Campylobacter* and *Escherichia coli* from animals (Aarestrup & Wegener, 1999). Antimicrobial resistance in zoonotic foodborne pathogens increases the burden of disease in humans by causing excess cases of illness and increasing morbidity and mortality among cases (Tollefson & Karp, 2004). The duration of illness was longer for patients infected with quinolone-resistant *Campylobacter* than those infected with susceptible strains (Engberg *et al*., 2004). Both pork and chicken are major sources of protein in Thailand, making the presence of resistant bacteria in these food animal species a potential source of zoonotic disease.

Antimicrobial use in both humans and animals may be the most important factor for the development of bacteria with increased resistance and virulence (Dancer, 2004). Bacteria exposed to antimicrobial agents may evolve to maintain survival, and the extent of resistance depends on the amount, duration, and interaction of antimicrobial agents and the bacterium (Yan & Gilbert, 2004). Modern food animal production uses large amounts of antimicrobial agents at subtherapeutic levels for growth promotion, which provides favorable conditions for the spread and persistence of antimicrobial-resistant zoonotic bacteria such as *Campylobacter* (Aarestrup & Wegener, 1999). The use of antimicrobials in animals in Thailand was only regulated by the Thai Department of Livestock Development in 2003, and not all farms in the country have met these new standards for antimicrobial drug use. The use of antimicrobial agents is unrestricted in Thailand, and many people will treat diarrhea themselves with antimicrobial drugs.

The prevalence of antimicrobial-resistant *Campylobacter* in our study, particularly the prevalence of resistance to fluoroquinolones, was relatively high compared to studies conducted in the US (Gupta *et al*., 2004), Europe (Bywater

Table 5. Comparison of patterns of antimicrobial resistance in *Campylobacter coli* isolated from chicken and pig fecal samples collected at the farm, 2000–2001

	Percent resistant by host		Mantel–Haenszel		
Agent	Chicken ($n = 92$)	Pig ($n = 51$)	χ^2	*P*	Fisher's exact two-tailed *P*
Azithromycin	9.8	54.9	34.6	<0.0001	<0.0001
Chloramphenicol	0	2.0	*	*	0.3506
Ciprofloxacin	87.0	86.3	0.01	0.9087	1.0
Clindamycin	8.7	64.7	50.0	<0.0001	<0.0001
Erythromycin	8.7	56.9	39.4	<0.0001	<0.0001
Gentamicin	0	13.7	*	*	0.0006
Nalidixic acid	89.1	90.2	0.04	0.8426	1.0
Tetracycline	51.1	88.2	19.6	<0.0001	<0.0001
Multiresistant	50.0	92.2	25.5	<0.0001	<0.0001

*χ^2 test not valid due to low numbers of samples.

et al., 2004), and Japan (Ishihara *et al*., 2004). Several factors may account for these differences. While it has been suggested that antimicrobial use in food animal production may increase antimicrobial-resistant *Campylobacter* (Aarestrup & Wegener, 1999), other researchers have found that fluoroquinolone-resistant *Campylobacter* may persist despite the use of fluoroquinolones (Zhang *et al*., 2003). The effects of antimicrobial use on the proportion of antimicrobial-resistant *Campylobacter* on farms is unclear; associations were found between organic (no/limited antimicrobial use) or conventional farm management and the proportion of resistance in a poultry farm in one study (Avrain *et al*., 2003), but no associations were seen in another study of organic and conventionally managed dairy farms (Sato *et al*., 2004).

The differences in patterns of antimicrobial resistance seen between different species in this study may be due to several factors. Since exposure to antimicrobial agents aids the development of resistance to specific agents in bacteria (Aarestrup & Wegener, 1999), differences in the types of drugs used to treat different food animal species would create preferential selection pressures for any enteric organisms present in the host. In the current study, chickens were exposed to drugs such as amprolium, sulfamethoxazole, and enrofloxacin prior to their entry on study farms. Pigs were given lincomycin and tylosin before they entered the study. Resistance to the fluoroquinolones ciprofloxacin and nalidixic acid was seen in *C. coli* from chickens in this study (Table 5). In the case of pigs, exposure to one macrolide (tylosin) may have resulted in the high levels of resistance to another macrolide (erythromycin) seen in this study (Table 5). Also, antimicrobial concentrations may vary in different segments of organs after administration, such that bacteria may be subjected to uncertain selection pressures depending on their location in the host (Yan & Gilbert, 2004).

In addition to differences in exposures to antimicrobial agents, there were differences in the proportions of species of *Campylobacter* (*C. jejuni* or *C. coli*) from different species groups. One study in Japan demonstrated that a higher proportion of *C. coli* than *C. jejuni* were resistant to aminoglycosides, macrolides, tetracycline and quinolones (Ishihara *et al*., 2004). The differences in antimicrobial susceptibility reported between *C. jejuni* and *C. coli* may account for some of the differences seen between isolates from pigs and chickens in this study.

In addition to the development of resistance, the spread of resistant bacteria throughout the human food chain is another concern, especially in cases where the proportions of multiresistant isolates and isolates resistant to ciprofloxacin and tetracycline were higher at the market than on the farm or at slaughter (Table 3). Finding the highest levels of antimicrobial resistance in samples from markets suggests that, in addition to resistant *Campylobacter* arriving at the market directly from the animals, cross-contamination may be occurring at the marketplace itself. This is particularly worrisome for samples from pork at the market, where over 50% of *Campylobacter* from these samples were multiresistant. The practice of leaving raw pork and chicken in the open air at the market would facilitate cross-contamnation from environmental contaminants or from humans working or shopping at the market. Measures could be taken at the market to attempt to reduce cross-contamination to reduce the spread of resistant isolates or transmissible resistance factors.

In conclusion, this study has provided information about the prevalence of antimicrobial resistance in *Campylobacter* from food animals at different stages in the chain from farm to market, and from healthy adults and children with diarrhea in Thailand. There were significant differences in the prevalence of resistant *Campylobacter* among animals at the farm for all agents tested, and between sampling locations for most agents tested. The increased prevalence of resistant isolates from meat samples collected at market, compared to isolates collected from animals on the farm or at the slaughterhouse, suggests that contamination of foods of animal origin after carcasses leave the slaughterhouse is an important factor in the spread of resistant bacteria to the human food chain. Tracking changes in antimicrobial susceptibility in *Campylobacter* from food animals and food of animal origins was beyond the scope of this study; however, these findings indicate areas where future research can be targeted to identify specific factors to reduce the prevalence of resistant bacteria entering the human food supply.

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