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Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China

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ABSTRACT

The prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler chickens were determined in Shandong Province, China. In total, 275 *Campylobacter* isolates were obtained from 767 broiler cecal samples, including 208 *Campylobacter jejuni*, 53 *Campylobacter coli*, and 14 unidentified *Campylobacter* isolates. Minimal inhibitory concentrations of 11 antimicrobial agents were determined using the agar dilution method recommended by CLSI. More than 98% of the tested *Campylobacter* isolates were resistant to quinolones (nalidixic acid, ciprofloxacin and enrofloxacin) and tetracyclines (tetracycline and doxycycline). The *C. jejuni* isolates also exhibited a high rate of resistance to phenicol antibiotics and a moderate rate of resistance to macrolides and gentamicin. On the contrary, the *C. coli* isolates showed a high-level resistance to macrolides and gentamicin and little resistance to phenicol antibiotics. The vast majority of the *Campylobacter* isolates were classified as multidrug resistant. These findings reveal a broad extent of antimicrobial resistance in *Campylobacter* isolates from poultry in China and underline the need for prudent use of antibiotics in poultry production to minimize the spread of antibiotic resistant *Campylobacter*.

1. Introduction

Thermophilic *Campylobacter*, including *Campylobacter jejuni* and *Campylobacter coli*, is a main bacterial cause of acute gastroenteritis in humans in both developing and developed countries (Blaser, 1997; Englen et al., 2007). *Campylobacter* infection is also associated with the development of Guillain-Barré syndrome, a neurological disorder affecting the peripheral nervous system (Yuki, 2001; Leonard et al., 2004). For clinical treatment of campylobacteriosis, macrolide and fluoroquinolone antibiotics are often prescribed, however, *Campylobacter* resistance to both classes of antibiotics is on the rise (Payot et al., 2006; Gibreel and Taylor, 2006).

As a foodborne pathogen, *Campylobacter* is transmitted to humans via contaminated food and water (Allos, 2001). Particularly, the chicken is a natural host of *C. jejuni* and serves as a major reservoir for this pathogenic organism (Sahin et al., 2002; Lee and Newell, 2006). Contamination of chicken carcasses by *Campylobacter* often occurs during the slaughtering process and consumption of chicken meat is a significant source of human *Campylobacter* infections (Humphrey et al., 2007). Thus control of *Campylobacter* in poultry should yield a positive impact on improving food safety.

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For modern poultry production, antimicrobial agents have been widely used for growth promotion and disease control. Many of the antimicrobials used for animal agriculture are also used for human medicine. Thus, agricultural use of antibiotics poses a risk for selecting antibiotic resistant pathogens that can be potentially transmitted to humans and may compromise clinical treatment. Indeed, previous studies have shown that use of certain antimicrobials in chickens, especially fluoroquinolones, rapidly select for antibiotic-resistant *Campylobacter* (McDermott et al., 2002; Luo et al., 2003). Many studies have reported the prevalence of antimicrobial-resistant *Campylobacter* in animal reservoirs in different countries (Bachoual et al., 2001; Gibreel and Taylor, 2006; Alfredson and Korolik, 2007; Hariharan et al., 2009; Luangtongkum et al., 2009). However, little information is available on the prevalence and antimicrobial resistance of *Campylobacter* from poultry in China, where poultry production represents an important sector of animal husbandry and consumption of poultry meat is significant. In this study, we surveyed several broiler slaughter houses in five different regions of Shandong Provinces and determined the prevalence and antimicrobial resistance of *Campylobacter* in multiple chicken flocks.

2. Materials and methods

2.1. Isolation and Identification of Campylobacter

Campylobacter strains were isolated from cecal contents of broiler chickens, which were selected randomly from five different geographical areas in Shandong Province, China. All samples were collected in June 2008 from five slaughterhouses located in the southeast (Linyi), north (Zouping and Penglai), northwest (Longkou), and west (Shenxian) of Shandong Province. The samples were collected from 45 flocks (Table 1). From each flock, 15 up to 20 samples were collected.

The collected ceca were individually packed and transported on ice to the laboratory within 5 hours of collection. For each cecum, a loopful of the fecal content was directly streaked onto *Campylobacter* Selective Agar (Base) (Oxoid Ltd., Basingstoke, England) containing 5% fresh sterile defibrinated sheep blood and *Campylobacter* supplement III (Sigma, St. Louis, MO, USA) for primary isolation. The plates were incubated in an environment of 10% CO_2, 5% O_2 and 85% N_2 at 42 °C for 36–48 h. One suspected colony was isolated from each cecal sample.

The isolates were identified to the genus/species level by multiplex PCR with three pairs of primers amplifying the 16S rRNA gene specific for the genus of *Campylobacter* (Linton et al., 1997), the *HipO* gene specific for *C. jejuni*, and the CC amplicon (located in the 16S–23S rRNA region) specific for *C. coli* (Keramas et al., 2003). The primers were listed in Table 2.

For PCR, crude chromosomal DNA of the isolates was prepared by boiling as described previously (Bachoual et al., 2001). The PCR mixture consisted of 10 μL of 2× PCR MasterMix (TIANGEN, Beijing, China), 0.4 μL of 10 nmol/L of each primer, 1 μL of chromosomal DNA template, and 6.6 μL of sterile distilled water. The PCR was carried out in a Veriti 96 well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with the following cycling conditions: heat denaturation at 95 °C for 5 min, 35 cycles at 95 °C for 35 s, 56.5 °C for 40 s, 72 °C for 45 s, and a final extension at 72 °C for 7 min. The *C. jejuni* ATCC 33560™ and *C. coli* ATCC 33559™ strains obtained from the American Type Culture Collection (Manassas, VA, USA) were used as the positive control.

2.2. Antimicrobial susceptibility testing of Campylobacter isolates

The agar dilution method was used to determine the susceptibility of *Campylobacter* isolates to 11 antimicrobial

Table 1
Campylobacter isolates obtained from broiler cecal samples.

Region	Number of samples	Number of flock	Number of positive flocks[a]	Number of *C. jejuni*[a]	Number of *C. coli*[a]	Number of unidentified *Campylobacter* species[a]	Total number of positive samples from each region[a]
Zouping	82	4	3 (75)	12 (14.6)	0 (0)	0 (0)	12 (14.6)
Linyi	370	23	16 (70.0)	100 (27)	3 (0.2)	0 (0)	103 (27.8)
Shenxian	185	11	9 (81.8)	71 (38.4)	16 (8.6)	7 (3.8)	94 (50.8)
Penglai	86	4	4 (100)	19 (22.1)	32 (37.2)	7 (8.1)	58 (67.4)
Longkou	44	3	3 (100)	6 (13.6)	2 (4.5)	0 (0)	8 (18.2)
Total number (%)	767	45	35 (77.7)	208 (27.1)	53 (6.9)	14 (1.8)	275 (35.9)

[a] Numbers in parentheses indicate the percentages.

Table 2
Primers used for PCR identification in this study.

Primer	Primer Sequence (5′–3′)	Amplified gene	Position	Size (bp)
16S rRNAf	GCGAAGAACCTACCYGGRCTTGATA	16S rRNA; genus-specific	nt 948–1244 of the 16S rRNA gene	314
16S rRNAr	TCGCGRTATTGCGTCTCATTGTATATG			
hipOf	GTACTGCAAAATTAGTGGCG	*hipO* of *C. jejuni*	nt 1478–1513 of the *hipO* gene	149
hipOr	GCAAAGGCAAAGCATCCATA			
CCf	GTTAAGAGTCACAAGCAAGT	Intergenic region between 16S and 23S rRNA; *C. coli* specific	nt 488–523 of the *C. coli* 16S and 23S rRNA intergenic spacer region	194
CCr	CTAAAAATATCTAAACTAAGTCG			