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Short communication

Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China

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ABSTRACT

This study was conducted to determine the prevalence and distribution of *Campylobacter* species along a broiler production chain from farm to retail, and to evaluate the antimicrobial resistance profile of *Campylobacter* isolates. A total of 259 *Campylobacter* isolates (*C. jejuni* n = 106, *C. coli* n = 153) were isolated from broiler ceca samples (72.5%, 103/142), broiler carcasses (34.1%, 46/135), and retail broiler meat (31.3%, 40/128) samples collected in Shanghai, China. Minimal inhibitory concentrations of six antimicrobials were determined using the agar dilution method. High prevalence of resistance to ciprofloxacin (*C. jejuni*: 99.1%;*C. coli*: 100%) and tetracycline (*C. jejuni*: 100%;*C. coli*: 98.7%) was detected among the *C. jejuni* and *C. coli* isolates. The vast majority of *C. coli* were resistant to clindamycin (92.2%), gentamicin (95.4%), and erythromycin (94.1%), but only 25.5%, 53.8%, and 16.0% of *C. jejuni* exhibited resistance to these three antimicrobials, respectively. In contrast, the prevalence of florfenicol resistance in *C. jejuni* (37.7%) was significantly higher than that in *C. coli* (7.8%) ($P < 0.05$). It is noteworthy that all *Campylobacter* isolates were resistant to one or more antimicrobials, and 71.7% of *C. jejuni* and 98.0% of *C. coli* isolates exhibited multi-drug resistance (resistant to three or more antimicrobials). Fifty-five *C. jejuni* and sixty *C. coli* isolates, selected from different production stages, species, and antimicrobial resistance patterns, were analyzed by pulsed field gel electrophoresis (PFGE), among which 15 unique PFGE patterns (PFGE patterns represented by a single strain) and 31 clusters (PFGE patterns represented by multiple strains) were detected. Furthermore, nearly all of the PFGE patterns of the *Campylobacter* strains isolated from retail broiler meats overlapped with those of the strains from ceca and slaughterhouse carcasses. Together, these findings revealed the high prevalence of *Campylobacter* species in a broiler chicken production chain, and the concerning situation of antimicrobial resistance in *Campylobacter* species. The findings also indicated that *Campylobacter* isolates from retail broiler meats were associated with fecal contamination in the slaughterhouse, underlying the need for improved measures for reducing carcass contamination in slaughter plants.

1. Introduction

Campylobacter species, especially *C. jejuni* and *C. coli*, are the most frequently identified foodborne bacteria causing gastroenteritis throughout the world (Coker et al., 2002). Although most campylobacteriosis occurs as self-limiting enteritis, more severe and long-lasting cases, particularly in immune-comprised patients, may require antibiotic treatment (Gibreel et al., 2004). The macrolides erythromycin and azithromycin are the antimicrobials of choice when therapeutic intervention is warranted. Other antibiotic options include fluoroquinolones (ciprofloxacin), aminoglycosides (gentamicin), and tetracyclines (Wardak et al., 2007). *Campylobacter* species are increasingly resistant to these clinically important antibiotics, which compromises clinical therapy and presents a major threat to public health (Anderson et al., 2001; Cox and Popken, 2006). Use of antimicrobials in animal husbandry contributes to the selection of antibiotic resistant *Campylobacter* strains that are transmitted to humans through the food chain (Radostits and Rubinstein, 2002).

Epidemiological studies have demonstrated that handling and consumption of contaminated poultry meat, particularly chicken products, are a major source of human *Campylobacter* species infections (Samuel et al., 2004; Wingstrand et al., 2006). Broiler chickens have been regarded as one of the main reservoirs of *Campylobacter* species, and the colonization level of *Campylobacter* species in broiler ceca can reach as high as 10^9 CFU/g (Stern et al., 2008). Carcass contamination usually occurs directly via leakage of intestinal contents during the slaughtering process (Elvers et al., 2011). Studies have been conducted to investigate the prevalence and antimicrobial susceptibility of poultry-associated *Campylobacter* species, and most of them have

Abbreviations: PFGE, pulsed field gel electrophoresis; MIC, minimum inhibitory concentration; QC, quality control; MDR, multi-drug resistance; "F", Florfenicol; "C", Ciprofloxacin; "T", Tetracycline; "L", Clindamycin; "G", Gentamicin; "E", Erythromycin.

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Table 1
Antimicrobial testing ranges, MIC QC ranges, and breakpoints used for antimicrobial susceptibility testing by agar dilution for *Campylobacter* species.

Antimicrobial agents	Test ranges (μg/mL)[a]	MIC QC ranges (μg/mL)	MIC breakpoints (μg/mL)[b]		
			S	I	R
Florfenicol	0.06–128	0.25–2	≤4	8	≥16
Ciprofloxacin	0.03–512	0.06–0.5	≤1	2	≥4
Tetracycline	0.06–512	0.25–1	≤4	8	≥16
Clindamycin	0.03–512	0.125–0.5	≤2	4	≥8
Gentamicin	0.06–512	0.5–4	≤2	4	≥8
Erythromycin	0.06–512	1–8	≤8	16	≥32

[a] Agar dilution QC ranges of *C. jejuni* ATCC33560 approved by the CLSI (2008).
[b] MIC breakpoints used in this study are those recommended by the CLSI (2008). S, susceptible; I, intermediate; R, resistant.

reported the emergence of resistant strains (Andersen et al., 2006; Osaili et al., 2011). In China, an active surveillance system to monitor the prevalence and antimicrobial resistance of *Campylobacter* species from farm to retail is not yet available, and little information has been reported on *Campylobacter* species in chicken (Chen et al., 2010). The prevalence and distribution of *Campylobacter* species along the broiler production chain are unknown in China, which hamper the implementation of interventions.

To collect information concerning *Campylobacter* in poultry production in China, this study tracked *Campylobacter* contamination in ceca, carcasses, and retail meats of a broiler production chain in Shanghai, China. The isolates were profiled for antimicrobial susceptibility, and PFGE was employed to explore the diversity and linkage of *Campylobacter* species isolates from different production stages.

2. Materials and methods

2.1. Sample collection

The investigation was conducted during October and November of 2012 in a vertically-integrated commercial poultry production continuum in Shanghai, China, in which more than 1,000,000 broiler chickens were reared, slaughtered, and sold per year. A total of 142 broiler cecal samples (one from each broiler), representing samples of broiler at the farm level, were collected at a slaughterhouse after evisceration. One hundred and thirty-five whole broiler carcasses were also sampled at the end of the processing steps (after chilling) in the slaughterhouse. Furthermore, 128 whole broiler chicken carcasses were randomly collected from two supermarkets. All of the samples were transported to the laboratory on ice within 3 h of collection and were analyzed immediately. Although the samples collected from the three stages were probably derived from different broiler flocks, they belonged to the same broiler production chain from farm to retail.

2.2. Isolation and Identification of Campylobacter species

For each cecal sample, a loopful of fecal material was directly streaked onto a *Campylobacter* selective agar plate (Sigma, St. Louis, MO, USA) containing 5% fresh sterile defibrinated sheep blood and *Campylobacter* supplement III (Sigma), and then incubated at 42 °C in a microaerophilic chamber (10% CO_2, 5% O_2, 85% N_2) for 48–72 h (Chen et al., 2010).

Each whole broiler carcass obtained from the slaughterhouse or supermarkets was put into a sterile bag containing 100 mL of nutrient broth no. 2 (CM0067; Oxoid, Basingstoke, UK). The carcass was massaged for 1 min, then 25 mL of the rinsate was added to 225 mL of Preston broth (nutrient broth no. 2 CM0067, *Campylobacter* selective supplement SR0117E, and *Campylobacter* growth supplement SR0232E; Oxoid) containing 5% defibrinated sheep blood. The broth was then incubated for 4 h at 37 °C, and an additional 44 h at 42 °C under microaerophilic conditions. A loopful of broth was then streaked onto a *Campylobacter* selective agar plate and incubated for 48–72 h at 42 °C under microaerophilic conditions. For each positive plate, up to three presumptive *Campylobacter* colonies were selected for further identification using multiplex PCR, as previously described (Keramas et al., 2003), and API-Campy kits (BioMerieux, Marcy l'Etoile, France). A primer set specific for the *C. jejuni* hippuricase gene and the primer sets for the 16S/23S rRNA internal regions were used in the multiplex PCR to identify *C. jejuni* and *C. coli*, respectively. Where isolates from the same sample, only one of the isolates that belonged to the same species and had the same antibiotic resistance pattern was selected for the subsequent analysis. All confirmed isolates were stored at −80 °C in Brain Heart Infusion broth (Land-bridge, Beijing, China) containing 20% (v/v) glycerol.

2.3. Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using a standard agar dilution method in Mueller–Hinton agar supplemented with 5% sheep blood, as described by the Clinical and Laboratory Standards Institute (CLSI, 2008). The agar plates were incubated at 42 °C for 24 h under a microaerophilic atmosphere. The following antimicrobial agents were used: florfenicol, ciprofloxacin, tetracycline, clindamycin, gentamicin, and erythromycin. All the antimicrobial agents were obtained from the China Institute of Veterinary Drug Control (Beijing, China). *C. jejuni* ATCC33560 was used as the quality control organism. Breakpoints for each antimicrobial agent and quality control (QC) minimum inhibitory concentration (MIC) ranges with ATCC 33560 are shown in Table 1.

2.4. Pulsed field gel electrophoresis (PFGE)

PFGE analysis was performed as previously described (Ribot et al., 2001), using *Sma*I as the restriction endonuclease and *Salmonella* H9812 as the reference marker (digested with *Xba*I) (Hunter et al.,

Table 2
Prevalence of *Campylobacter* species isolated from ceca, slaughterhouse carcasses, and retail broiler meats in Shanghai, China.

Samples	No. (%) of positive samples			No. of *Campylobacter* isolates	
	C. jejuni	*C. coli*	*C. jejuni* + *C. coli*	*C. jejuni*	*C. coli*
Broiler ceca	40 (28.2%, 40/142)	46 (32.4%, 46/142)	17 (12.0%, 17/142)	69	88
Slaughterhouse carcasses	14 (10.4, 14/135)	24 (17.8%, 24/135)	8 (5.9%, 8/135)	23	33
Retail broiler meats	9 (7.0%, 9/128)	28 (21.9%, 28/128)	3 (2.3%, 3/128)	14	32
Total	63 (15.6%, 63/405)	98 (24.2%, 98/405)	28 (6.9%, 28/405)	106	153