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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

2005). PFGE results were analyzed using the InfoQuest FP software version 4.5 (Bio-Rad Laboratories), and the banding patterns were clustered using Dice coefficients with a 2% band position tolerance and 1.5% optimization. A PFGE pattern was defined as a group of strains with a Dice coefficient similarity of 85% or greater, and the PFGE pattern represented by multiple strains was a PFGE cluster.

2.5. Statistical analysis

Prevalence and frequency of antimicrobial resistance profiles of *Campylobacter* isolates obtained from different stages were compared using the Chi-square test in the statistical software SPSS 17.0 (SPSS Inc, Chicago, IL, USA). Differences were considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Incidence of Campylobacter species

Overall, 103 of the 142 (72.5%) cecal samples tested positive (Table 2) for *Campylobacter* species, and 17 of the 142 (12.0%) positive samples were positive for both *C. jejuni* and *C. coli*, indicating the high prevalence of *Campylobacter* in broiler ceca. Considering that only three colonies were picked from each positive plate, the real positive rate for both species might be even higher. A total of 157 *Campylobacter* isolates were obtained from ceca, including 69 *C. jejuni* and 88 *C. coli*. Among the 135 broiler carcasses from the slaughterhouse, 46 (34.1%) samples tested positive for *Campylobacter* species (Table 2). A total of 56 *Campylobacter* isolates were acquired, including 23 *C. jejuni* and 33 *C. coli* isolates. For the 128 broiler

Table 3
Distribution of MIC for six antimicrobials in *C. jejuni* isolated from ceca, slaughterhouse carcasses, and retail broiler meats in Shanghai, China.

Antimicrobials	Samples	Distribution (No. of isolates) of MIC (μg/mL)[a]												MIC_{50}/MIC_{90}	Resistant
		≤0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	(μg/mL)	isolates[b]
Florfenicol	Broiler ceca	0	0	1	5	8	29	23	2	1	0	-[c]	-[c]	8/16	26(37.7%)
	Slaughterhouse carcasses	0	0	0	4	3	6	2	4	4	0	-[c]	-[c]	8/64	10(43.5%)
	Retail broiler meats	0	0	0	2	4	4	4	0	0	0	-[c]	-[c]	8/16	4(28.6%)
Ciprofloxacin	Broiler ceca	0	0	0	0	0	1	0	2	18	21	25	2	128/256	69(100%)
	Slaughterhouse carcasses	0	0	0	1	0	0	6	3	6	3	4	0	64/256	22(95.7%)
	Retail broiler meats	0	0	0	0	0	0	0	3	2	3	6	0	128/256	14(100%)
Tetracycline	Broiler ceca	0	0	0	0	0	0	1	3	11	5	31	18	256/512	69(100%)
	Slaughterhouse carcasses	0	0	0	0	0	0	0	2	7	3	8	3	128/512	23(100%)
	Retail broiler meats	0	0	0	0	0	0	0	3	0	0	5	6	256/512	14(100%)
Clindamycin	Broiler ceca	0	3	15	22	7	6	4	2	0	1	0	9	2/512	22(31.9%)
	Slaughterhouse carcasses	7	5	7	0	0	1	1	1	0	0	0	1	0.5/16	4(17.4%)
	Retail broiler meats	1	3	7	2	0	0	0	1	0	0	0	0	1/2	1(7.1%)
Gentamicin	Broiler ceca	1	10	9	0	0	0	0	0	0	11	12	26	256/512	49(71.0%)
	Slaughterhouse carcasses	5	8	3	3	1	0	0	0	0	1	2	0	0.5/128	3(13.0%)
	Retail broiler meats	3	4	1	1	0	0	0	0	1	1	0	3	0.5/512	5(35.7%)
Erythromycin	Broiler ceca	0	13	15	11	2	8	7	0	0	3	5	5	2/256	13(18.8%)
	Slaughterhouse carcasses	0	2	14	2	2	0	0	0	0	0	2	1	1/256	3(13.0%)
	Retail broiler meats	0	0	4	6	2	1	0	0	0	0	0	1	2/8	1(7.1%)

[a]Thin vertical lines indicate the breakpoint between susceptible and intermediate isolates. Thick vertical lines indicate the breakpoint between intermediate and resistant isolates.
[b]Numbers in parentheses indicate the percentages.
[c] No data available.

Table 4
Distribution of MIC for six antimicrobials in *C. coli* isolated from ceca, slaughterhouse carcasses, and retail broiler meats in Shanghai, China.

Antimicrobials	Samples	Distribution (No. of isolates) of MIC (μg/mL)[a]												MIC_{50}/MIC_{90}	Resistant
		≤0.25	0.5	1	2	4	8	16	32	64	128	256	≥512	(μg/mL)	isolates[b]
Florfenicol	Broiler ceca	1	0	6	58	11	6	5	0	1	0	-[c]	-[c]	2/8	6(6.8%)
	Slaughterhouse carcasses	0	0	0	8	10	9	5	0	1	0	-[c]	-[c]	4/16	6(18.2%)
	Retail broiler meats	0	0	0	20	10	2	0	0	0	0	-[c]	-[c]	2/4	0(0.0%)
Ciprofloxacin	Broiler ceca	0	0	0	0	1	0	0	11	32	37	6	1	64/128	88(100%)
	Slaughterhouse carcasses	0	0	0	0	0	0	9	3	11	4	6	0	64/256	33(100%)
	Retail broiler meats	0	0	0	0	0	0	1	1	2	17	10	1	128/256	32(100%)
Tetracycline	Broiler ceca	0	0	0	1	0	1	2	35	31	8	7	3	16/256	86(97.7%)
	Slaughterhouse carcasses	0	0	0	0	0	0	0	8	1	8	12	4	128/512	33(100%)
	Retail broiler meats	0	0	0	0	0	0	0	16	1	3	11	1	32/256	32(100%)
Clindamycin	Broiler ceca	0	0	5	2	0	0	2	2	0	1	0	76	512/512	81(92.0%)
	Slaughterhouse carcasses	1	0	0	0	2	4	5	13	0	0	0	8	32/512	30(90.9%)
	Retail broiler meats	0	1	0	0	1	1	1	6	7	0	0	15	64/512	30(93.8%)
Gentamicin	Broiler ceca	0	2	2	1	0	0	0	0	1	6	41	35	256/512	83(94.3%)
	Slaughterhouse carcasses	1	0	0	0	0	0	0	1	4	7	10	10	256/512	32(97.0%)
	Retail broiler meats	0	1	0	0	0	0	0	0	2	3	16	10	256/512	31(96.9%)
Erythromycin	Broiler ceca	0	3	2	0	0	2	0	0	1	1	32	47	256/512	81(92.0%)
	Slaughterhouse carcasses	0	1	0	0	0	0	0	0	0	13	7	12	256/512	32(97.0%)
	Retail broiler meats	0	0	1	0	0	0	0	0	0	8	11	12	256/512	31(96.9%)

[a]Thin vertical lines indicate the breakpoint between susceptible and intermediate isolates; thick vertical lines indicate the breakpoint between intermediate and resistant isolates.
[b]Numbers in parentheses indicate the percentages.
[c]No data available.

carcass samples obtained from supermarkets, 40 (31.3%) samples tested positive for *Campylobacter* species and 59 *Campylobacter* isolates were obtained, among which 14 and 32 were identified as *C. jejuni* and *C. coli*, respectively (Table 2). The prevalence of *Campylobacter* species in retail broiler carcasses was lower than that in carcasses from the slaughterhouse, but the difference was not statistically significant ($P = 0.63$). After packaging, the broiler meats were immediately transported to supermarkets at 4 °C. The short time of exposure to air and favorable temperature conditions might help *Campylobacter* species to survive, which could explain the similar prevalence rates between slaughterhouse carcasses and retail carcasses.

Interestingly, this study found that *C. coli* (59.1%, 153/259) was more prevalent than *C. jejuni* (40.9%, 106/259) (Table 2). Two recent investigations by our laboratory carried out in Shandong and Henan Provinces, China, in 2011 and 2013 also showed similar findings (unpublished data). Similar results, showing that *C. coli* was the predominant *Campylobacter* species in broiler intestinal tracts, were also reported in Grenada (Hariharan et al., 2009) and Reunion Island (Henry et al., 2011). However, the higher prevalence of *C. coli* in broiler chickens is contrary to the findings in an earlier study conducted in Shandong Province (Chen et al., 2010) and in many reports from other countries, such as Hungary (Damjanova et al., 2011), Ethiopia (Ewnetu and Mihret, 2010), and Korea (Kang et al., 2006),

Table 5
Antimicrobial resistance rates of *Campylobacter* species isolated from the broiler production chain.

Antimicrobials	Antimicrobial resistance rates of tested *Campylobacter* isolates % (n)[a]	
	C. jejuni % (N = 106)[b]	*C. coli* % (N = 153)[b]
Florfenicol	37.7%(40)	7.8%(12)
Ciprofloxacin	99.1%(105)	100%(153)
Tetracycline	100%(106)	98.7%(151)
Clindamycin	25.5%(27)	92.2%(141)
Gentamicin	53.8%(57)	95.4%(146)
Erythromycin	16.0%(17)	94.1%(144)

[a] n, the number of resistant *Campylobacter* isolates.
[b] N, the number of *Campylobacter* isolates tested.

where *C. jejuni* was reported to be the predominant *Campylobacter* species in broiler chickens. The new information from our present work may indicate that *C. coli* is becoming the predominant *Campylobacter* species in chickens in China.

3.2. Antimicrobial susceptibility

All of the 259 *Campylobacter* isolates (106 *C. jejuni* and 153 *C. coli*) were subjected to antimicrobial susceptibility testing against six antimicrobial agents belonging to six different antimicrobial classes. The results are presented in Tables 3 and 4. All 259 *Campylobacter* isolates recovered from the broiler production chain were resistant to at least one of the tested antimicrobial agents. Notably, nearly all of the isolates were resistant to ciprofloxacin (*C. jejuni*: 99.1%;*C. coli*: 100%) and tetracycline (*C. jejuni*: 100%;*C. coli*: 98.7%) (Table 5). These results are consistent with a previous finding that nearly 100% of *Campylobacter* isolates from broiler chickens in China were resistant to ciprofloxacin and tetracycline (Chen et al., 2010). Moreover, the vast majority of *C. coli* isolates were also resistant to clindamycin (92.2%), gentamicin (95.4%), and erythromycin (94.1%). Furthermore, 121 of the 153 (79.1%) *C. coli* isolates exhibited high-level (MIC ≥ 256 μg/mL) erythromycin resistance. Compared to those of *C. coli*, the resistance rates of *C. jejuni* to clindamycin (25.5%), gentamicin (53.8%), and erythromycin (16.0%) were relatively low. The lowest resistance observed in these *Campylobacter* species was to florfenicol. Interestingly, the prevalence of florfenicol resistance in *C. jejuni* isolates (37.7%) was significantly higher than that in *C. coli* (7.8%) ($P < 0.05$). The variation in antimicrobial resistance between *C. jejuni* and *C. coli* might be attributed to their intrinsic ability to develop resistance to these antimicrobials (Chen et al., 2010). In addition, compared to *C. jejuni* from the other two sources, the isolates obtained from broiler cecal samples exhibited the highest resistance to clindamycin (31.9%), gentamicin (71.0%), and erythromycin (18.8%). The variation in prevalence of antimicrobial resistance among *Campylobacter* isolates derived from different production stages could be explained by the speculation that antibiotic resistance might has a fitness cost to the organism and therefore affects survival during the production process, which will be examined in future studies.

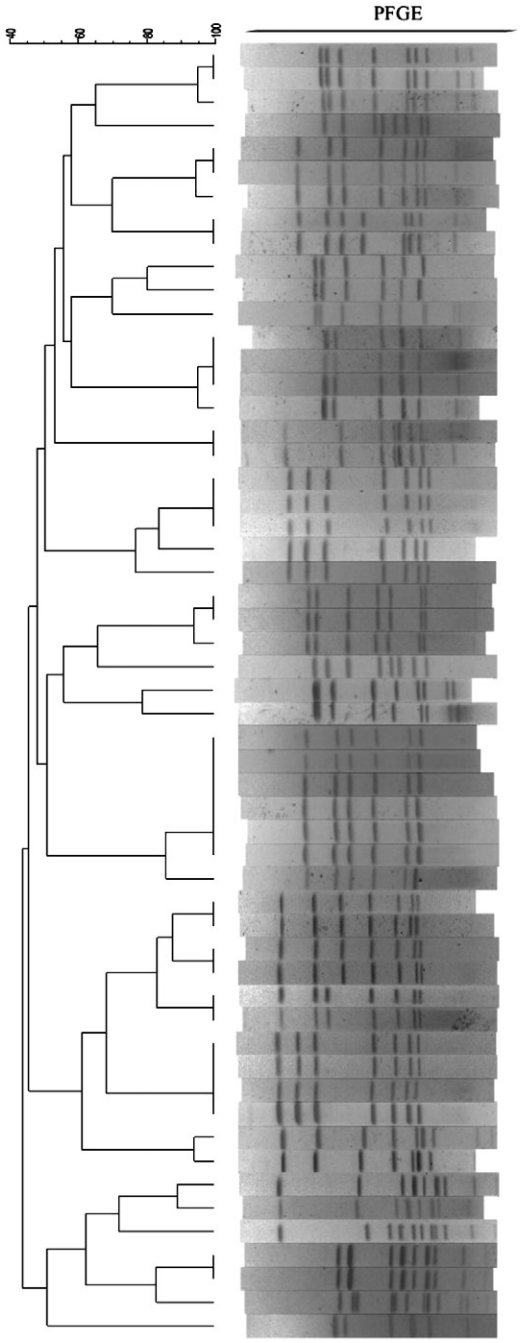
Antimicrobial resistance patterns of *C. jejuni* and *C. coli* isolates are outlined in Table 6. The 259 *Campylobacter* strains exhibited 15 antimicrobial resistance patterns. It is alarming that all but one of the *Campylobacter* isolates were resistant to two or more antimicrobials. Furthermore, 228 of the 259 (88.0%) *Campylobacter* isolates exhibited multi-drug resistance, higher than figures reported from other countries, including Northern Ireland (25.0%) (Wilson, 2003), France (37.0%) (Payot et al., 2004), Canada (29.7%) (Varela et al., 2007), and Korea (56.1%) (Shin and Lee, 2010). Considering resistance by species, multi-drug resistance was higher among *C. coli* (98.0%, 150/153) than *C. jejuni* (71.7%, 76/106), consistent with results reported by others (Noormohamed and Fakhr, 2012). For the *C. coli* isolates from all three production stages, there was a single predominant antimicrobial resistance pattern: C-T-L-G-E (resistant to ciprofloxacin, tetracycline, clindamycin, gentamicin, and erythromycin). In contrast, the antimicrobial resistance patterns of *C. jejuni* varied across the broiler production chain, and the major common patterns were C-T and F-C-T (Table 6).

In China, fluoroquinolones, tetracyclines, macrolides, and aminoglycosides are commonly used to treat and prevent bacterial diseases in poultry. Most of these antimicrobials are readily available to producers, who may not necessarily consult with veterinarians prior to their use. Hence, inappropriate use of antimicrobials likely occurs on farms. The high prevalence of antimicrobial resistance in *Campylobacter* species could be attributed to extensive use of antimicrobials in broiler chicken production, which select for the emergence and spread of antimicrobial resistant *Campylobacter* isolates. It is worth noting that a high prevalence of florfenicol resistance in *C. jejuni* was observed in this study. Florfenicol resistance in *Campylobacter* species is rarely reported worldwide (Ozawa et al., 2012; Perez-Boto et al., 2013), and the high prevalence in China might be caused by use of florfenicol in food-producing

Table 6
Antimicrobial resistance patterns of *Campylobacter* species isolated from the broiler production chain and the number of isolates selected for PFGE for each resistance patterns.

Antimicrobial resistance patterns[a]	*C. jejuni* (m = 55)[e]			*C. coli* (m = 60)[e]		
	Farm (n = 69)[b]	Slaughterhouse (n = 23)[b]	Supermarket (n = 14)[b]	Farm (n = 88)[b]	Slaughterhouse (n = 33)[b]	Supermarket (n = 32)[b]
F-C-T-L-G-E	2.9% (1)[e]	0	0	4.5% (0)[e]	18.2% (3)[e]	0
C-T-L-G-E	14.5% (2)[e]	8.7% (2)[e]	7.1% (1)[e]	**81.8%**[d] (14)[e]	**72.7%**[d] (15)[e]	**93.8%**[d] (19)[e]
F-C-T-L-G	5.8% (1)[e]	0	0	0	0	0
F-C-T-G	**21.7%**[d](3)[e]	4.3% (1)[e]	14.3% (2)[e]	1.1% (0)[e]	0	0
C-T-L-G	7.2% (3)[e]	0	0	1.1% (1)[e]	0	0
C-T-L-E	0	4.3% (1)[e]	0	3.4% (2)[e]	0	0
F-C-T-L	0	4.3% (0)[e]	0	0	0	0
C-T-G-E	1.4% (1)[e]	0	0	1.1% (1)[e]	6.1% (1)[e]	3.1% (0)[e]
C-L-G-E	0	0	0	1.1% (0)[e]	0	0
F-C-T	7.2% (2)[e]	**34.8%**[d](8)[e]	**21.4%**[d] (3)[e]	1.1% (0)[e]	0	0
C-T-G	17.4% (3)[e]	0	14.3% (2)[e]	2.3% (0)[e]	0	0
C-T-L	1.4% (0)[e]	0	0	0	0	0
C-L-G	0	0	0	1.1% (1)[e]	0	0
C-T	**20.3%**[d] (5)[e]	**39.1%**[d] (7)[e]	**42.9%**[d] (6)[e]	1.1% (1)[e]	3.0% (1)[e]	3.1% (1)[e]
T	0	4.3% (1)[e]	0	0	0	0
MDR(%)[c]	71.7%			98.0%		

[a] abbreviations of antimicrobial agents: F, florfenicol; C, ciprofloxacin; T, tetracycline; L, clindamycin; G, gentamicin; E, erythromycin.
[b] The number of *Campylobacter* isolates.
[c] The multi-drug resistance rates of *C. jejuni* and *C. coli*isolates from the broiler production chain.
[d] The bold text indicates the major resistance patterns (>20%).
[e] Number in parentheses indicate the number of *Campylobacter* isolates selected for PFGE analysis for each antimicrobial resistance pattern.



Strains	Sources	Resistance patterns	PFGE
CD133-1	Slaughterhouse	F-C-T	a
CM37-2	Supermarket	C-T	a
CM37-1	Supermarket	F-C-T	a
CF-C1	Farm	C-T	b
CD131-3	Slaughterhouse	F-C-T	c
CF29-1	Farm	F-C-T	c
CF-C12	Farm	C-T	c
CF202-4	Farm	C-T-G-E	d
CF230-2	Farm	C-T	d
CD133-3	Slaughterhouse	F-C-T	e
CM50	Supermarket	C-T	e
CD6	Slaughterhouse	F-C-T-G	f
CF107-2	Farm	C-T-L-G	g
CF115-2	Farm	C-T-L-G	g
CF157-2	Farm	F-C-T-L-G	g
CM99	Supermarket	F-C-T-G	g
CF232-1	Farm	C-T-L-G	h
CF68-1	Farm	C-T	h
CD131-2	Slaughterhouse	C-T	i
CD132-4	Slaughterhouse	C-T	i
CD90	Slaughterhouse	C-T	i
CM37-4	Supermarket	C-T	j
CD101-1	Slaughterhouse	F-C-T	j
CF109-1	Farm	C-T-L-G-E	k
CF210-1	Farm	C-T-L-G-E	k
CM28-3	Supermarket	C-T	k
CD132-1	Slaughterhouse	F-C-T	l
CM4-3	Supermarket	C-T-G	m
CM43-2	Supermarket	C-T-G	m
CF160-2	Farm	C-T-G	n
CF165-2	Farm	C-T-G	n
CF175-1	Farm	F-C-T-L-G-E	n
CF180-1	Farm	F-C-T-G	n
CF223-1	Farm	C-T-G	n
CF37-1	Farm	F-C-T-G	n
CM17	Supermarket	F-C-T-G	n
CM29-2	Supermarket	F-C-T	o
CM47	Supermarket	C-T	o
CD104-1	Slaughterhouse	F-C-T	o
CD126-2	Slaughterhouse	F-C-T	o
CD32	Slaughterhouse	T	p
CF13	Farm	C-T	p
CD117-1	Slaughterhouse	C-T	q
CD79	Slaughterhouse	C-T-L-E	q
CF183-1	Farm	F-C-T	q
CM38	Supermarket	C-T	q
CD104	Slaughterhouse	C-T	r
CM43-1	Supermarket	C-T-L-G-E	r
CD200-1	Slaughterhouse	C-T-L-G-E	s
CM91	Supermarket	F-C-T	s
CD124-3	Slaughterhouse	C-T-L-G-E	t
CD119-3	Slaughterhouse	C-T	u
CD140-2	Slaughterhouse	C-T	u
CD96-1	Slaughterhouse	F-C-T	v
CF129-2	Farm	F-C-T-G	w

Fig. 1. Dendrogram of *Sma*I PFGE patterns of 55 *C. jejuni* isolates. "Farm" indicates the isolates from cecal samples; "Slaughterhouse" indicates isolates from slaughterhouse carcasses; and "Supermarket" indicates the isolates from retail broiler meats. Abbreviations of antimicrobial agents: "F", Florfenicol; "C", Ciprofloxacin; "T", Tetracycline; "L", Clindamycin; "G", Gentamicin; and "E", Erythromycin;.

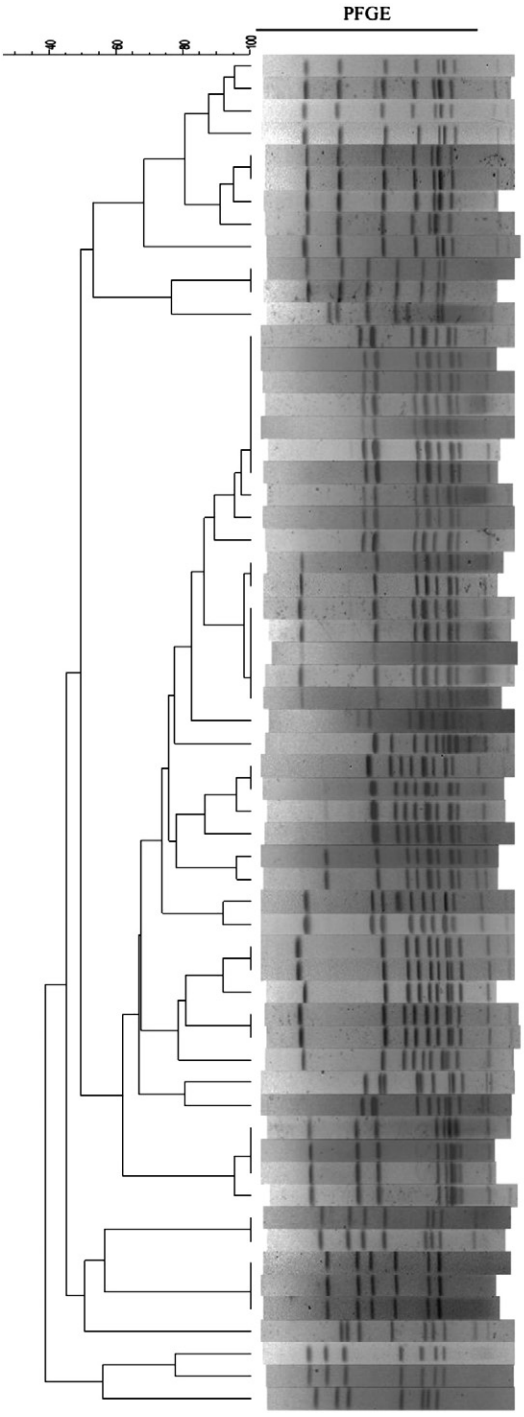
animals. Another important finding of this study is the extremely high prevalence of multidrug resistance in the *C. coli* isolates, which could explain why *C. coli* is replacing *C. jejuni* to become the predominant *Campylobacter* species in chickens in China. Thus, we speculate that *C. coli* is intrinsically more adaptable to antimicrobial treatment, and the extensive use of antimicrobials in broiler production may have favorably selected *C. coli* over *C. jejuni*.

3.3. PFGE patterns of Campylobacter isolates

A total of 55 *C. jejuni* and 60 *C. coli* isolates, representing isolates of different production stages, species, and antimicrobial resistance patterns, were selected for PFGE analysis (Table 6). The 55 *C. jejuni* isolates were grouped into 6 unique PFGE patterns (PFGE patterns represented by a single strain) and 17 clusters (PFGE patterns represented by multiple strains), while the 60 *C. coli* isolates were grouped into 9 unique PFGE patterns and 14 clusters. Slaughterhouse samples carried the most PFGE patterns (29 patterns), followed by retail samples (23 patterns), and cecal samples (19 patterns). With the exception of the strains with *C. jejuni* cluster m and unique pattern 15, and *C. coli* cluster 2 strains, which were only detected in retail samples, the PFGE patterns of most of the *Campylobacter* isolates from retail broiler meats were also found in strains from ceca or slaughter carcasses (Fig. 1 and Fig. 2), indicating that the majority of *Campylobacter* contamination of retail broiler meats occurred in the processing plants. For example, PFGE pattern q of *C. jejuni*, and PFGE patterns 6 and 10 of *C. coli* were found in all three

stages of the production chain. *Campylobacter* contamination of carcasses occurs during multiple steps of slaughtering, including defeathering, evisceration, and water chilling (Franchin et al., 2005). It has been suggested that the most effective mitigation strategy is a combination of chemical decontamination of carcasses and technical measures to reduce fecal contamination during slaughtering (Havelaar et al., 2005).

In summary, this study represents the first report describing the prevalence and antimicrobial resistance of *Campylobacter* species in a broiler chicken production chain in China. There is currently no information about the prevalence of *Campylobacter* species in humans in Shanghai, China. Thus, it will be very informative to investigate the relationship between *Campylobacter* species collected from animals and humans, and this topic requires further study. The high prevalence of *Campylobacter* in broiler chickens necessitates the implementation of improved intervention measures at the production and processing levels to minimize the occurrence of contamination. In addition, antimicrobial resistance was highly prevalent in *Campylobacter* species, and the majority of *Campylobacter* isolates were resistant to multiple antimicrobials, with high MIC values. The prevalence of antimicrobial resistance in *Campylobacter* species can be attributed to extensive use of antimicrobials in the broiler chicken production system in China. It



Stains	Sources	Resistance patterns	PFGE
CD103-2	Slaughterhouse	C-T-L-G-E	1
CM83	Supermarket	C-T-L-G-E	1
CD69	Slaughterhouse	C-T-L-G-E	1
CD53	Slaughterhouse	C-T-L-G-E	1
CM108	Supermarket	C-T-L-G-E	2
CM78	Supermarket	C-T-L-G-E	2
CM37-3	Supermarket	C-T-L-G-E	2
CM29-3	Supermarket	C-T-L-G-E	2
CD112-1	Slaughterhouse	C-T-L-G-E	3
CD116	Slaughterhouse	F-C-T-L-G-E	4
CM54	Supermarket	C-T-L-G-E	4
CF124-1	Farm	C-T-L-G-E	5
CD67-2	Slaughterhouse	C-T-L-G-E	6
CF101-1	Farm	C-T-L-G-E	6
CF111-2	Farm	C-T-L-G-E	6
CF154-1	Farm	C-T-L-G-E	6
CF98-1	Farm	C-T-L-G-E	6
CM52-1	Supermarket	C-T-L-G-E	6
CM80	Supermarket	C-T-L-G-E	6
CF170-1	Farm	C-T-L-G-E	6
CF123-1	Farm	C-T-L-G-E	6
CM107-1	Supermarket	C-T-L-G-E	6
CM28-2	Supermarket	C-T	7
CM94	Supermarket	C-T-L-G-E	7
CF157-1	Farm	C-L-G	7
CF163-2	Farm	C-T-L-G-E	7
CF182-1	Farm	C-T-L-G-E	7
CF189-1	Farm	C-T-L-G-E	7
CM120-1	Supermarket	C-T-L-G-E	7
CF144-1	Farm	C-T-L-G-E	8
CF12	Farm	C-T-L-G	9
CD80	Slaughterhouse	C-T-L-G-E	10
CF194-2	Farm	C-T-L-G-E	10
CM90-1	Supermarket	C-T-L-G-E	10
CF91-1	Farm	C-T-L-G-E	10
CF208-1	Farm	C-T-L-G-E	11
CM72-1	Supermarket	C-T-L-G-E	11
CD43-1	Slaughterhouse	C-T-L-G-E	12
CD78	Slaughterhouse	C-T-L-G-E	12
CD122	Slaughterhouse	F-C-T-L-G-E	13
CD98	Slaughterhouse	C-T-L-G-E	13
CM117-1	Supermarket	C-T-L-G-E	13
CD108-1	Slaughterhouse	C-T-L-G-E	14
CD126-1	Slaughterhouse	C-T-L-G-E	14
CM49	Supermarket	C-T-L-G-E	15
CD75	Slaughterhouse	F-C-T-L-G-E	16
CF-T11	Farm	C-T-G-E	17
CF-C4	Farm	C-T	18
CF-T3	Farm	C-T-L-E	18
CM44-2	Supermarket	C-T-L-G-E	18
CF-C3	Farm	C-T-L-E	18
CD90-2	Slaughterhouse	C-T-L-G-E	19
CM35	Supermarket	C-T-L-G-E	19
CD131-4	Slaughterhouse	C-T-G-E	20
CM123-1	Supermarket	C-T-L-G-E	20
CM126	Supermarket	C-T-L-G-E	20
CD87	Slaughterhouse	C-T-L-G-E	21
CD119-1	Slaughterhouse	C-T	22
CD49-3	Slaughterhouse	C-T-L-G-E	22
CD125-1	Slaughterhouse	C-T-L-G-E	23

Fig. 2. Dendrogram of *Sma*I PFGE patterns of 60 *C. coli* isolates. "Farm" indicates the isolates from cecal samples; "Slaughterhouse" indicates isolates from slaughterhouse carcasses; and "Supermarket" indicates the isolates from retail broiler meats. Abbreviations of antimicrobial agents: "F", Florfenicol; "C", Ciprofloxacin; "T", Tetracycline; "L", Clindamycin; "G", Gentamicin; and "E", Erythromycin;.

has been reported that resistant *Campylobacter* strains can be transmitted to humans through the food chain (Endtz et al., 1991; Hurd et al., 2008), which poses a serious risk for treatment failure. Given that contaminated broiler meat is the major source of human *Campylobacter* infections (Guerin et al., 2007), prudent measures for antimicrobial usage and active surveillance systems should be established to contain the prevalence and spread of antimicrobial resistant *Campylobacter* in chickens. Furthermore, most strains from retail broiler meats had the same antimicrobial resistance profiles and PFGE patterns as those from the previous stages of the production chain. These findings have important implications for the control of *Campylobacter* contamination in the food chain, and provide useful information for antimicrobial resistance risk assessment in poultry.

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