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Prevalence, antimicrobial resistance profiling and genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolated from broilers at slaughter in China

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ABSTRACT

A total of 651 samples from broiler cecal samples, carcasses, carcass parts collected at the slaughterhouse level in Sichuan Province of China were examined for the occurrence of *Campylobacter jejuni* and *Campylobacter coli*. After confirmed by species-specific multiplex PCR, the recovered isolates were examined for resistance to antimicrobials using an agar dilution method and investigated for the mutation of *gyrA*, *tetO* gene and V domain of 23S rRNA as well as the presence of class 1 integron and the associated gene cassettes. In addition, the genotype relatedness of the isolates was determined by pulsed-field gel electrophoresis (PFGE) profiling. The prevalence of *Campylobacter* was 56.1% in cecal samples, 31.0% in carcasses and 17.0% in carcass parts, respectively. Among them, *C. jejuni* accounted for 24.6% and *C. coli* occupied 20.0% of the samples. The strains of *C. jejuni* were most frequently resistant to ciprofloxacin (88.1%), followed by resistance to tetracycline (79.4%) and levofloxacin (78.1%). Most of the *C. coli* isolates were resistant to ciprofloxacin (100%), tetracycline (98.5%), levofloxacin (98.5%), clindamycin (98.5%) and erythromycin (93.9%). Antimicrobial resistance profiling showed that 93.7% of campylobacters were multidrug resistant (MDR) strains. Moreover, class 1 integrons were detected in 98.6% of MDR campylobacters, among which 98.7% were positive for *C. jejuni* and 98.5% for *C. coli*. Three kinds of gene cassettes-associated amplicons were identified and the amplicons profile of 1000–750–500–250 bp was the predominant pattern linked to the aminoglycoside resistance gene of *aadA2*. The presence of mutation in *gyrA*, *tetO* and 23S rRNA between *C. jejuni* and *C. coli* varied from 89.7% to 97.3%, 96.6% to 94.1%, and 95.0% to 96.7%, respectively. Finally, the results of PFGE indicated that, 33 PFGE profiles were generated among 78 isolates of *C. jejuni* and the similarity level ranged from 42.1% to 99.1%. By contrast, 15 PFGE patterns were produced among 68 *C. coli* isolates sharing a similarity level of 54.0%–99.7%. Comparison of the PFGE and antimicrobial resistance profiles of the isolates reflected the high genetic diversity of *Campylobacter* tested. A poor correlation among the antimicrobial resistance patterns, resistance determinants and PFGE genotypes was observed in *C. jejuni*. Our study showed that there were several points of cross-contamination during broiler slaughter, and a high diversity of PFGE types in the Campylobacter isolates with high resistances to ciprofloxacin and tetracycline.

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1. Introduction

Campylobacter species have been recognized as the most frequently identified bacterial causes of human gastroenteritis in many developed and developing countries (Iovine, 2013; Pepe et al., 2009; Taboada, Clark, Sproston, & Carrillo, 2013). Although

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mortality is low, morbidity imparted by post-infectious sequelae such as Guillain-Barré syndrome, Reiter syndrome/reactive arthritis and irritable bowel syndrome is significant (Humphrey, O'Brien, & Madsen, 2007; Iovine, 2013). The species most commonly associated with human infection is *Campylobacter jejuni* (80%–90%); followed by *Campylobacter coli* (5%–10%), but other *Campylobacter* species can also cause human infection (Rowe & Madden, 2014). Food animals, especially poultry, are the main reservoirs of *C. jejuni* and *C. coli* (Rasschaert, Houf, Van Hende, & De Zutter, 2006; Szczepanska et al., 2015).

Campylobacter cause little or no clinical diseases in poultry, but poultry carcasses have been frequently contaminated in the slaughterhouse due to the high prevalence of *Campylobacter* in the intestinal tract of market-age poultry and can be transferred to human via poultry carcasses (Ellerbroek, Lienau, & Klein, 2010; van Gerwe, Bouma, Wagenaar, Jacobs-Reitsma, & Stegeman, 2010). There are several identified sources of *C. jejuni* infection, such as consumption of raw milk, untreated water, foods, direct contact with pets and farm animals (Denis et al., 2009; Humphrey et al., 2007). Different studies of risk factors, however, point to the handling or consumption of chicken meat as the main source of infection in humans (Humphrey et al., 2007; Sproston, Carrillo, & Boulter-Bitzer, 2014). Widespread carcass contamination occurs in the slaughterhouse by direct contact with intestinal content or with dirty contact surfaces such as rubber fingers, conveyor belts and cutting tables (Melero, Juntunen, Hänninen, Jaime, & Rovira, 2012). Contaminated food and subsequent illness are a huge economic burden, and processing control and prevention measures are of great importance (Perez–Perez & Kienesberger, 2013).

In recent years, there is an increasing trend of antimicrobial resistance in *Campylobacter* isolates worldwide, especially multidrug resistant (MDR) strains within the food chain (Abdollahpour, Zendehbad, Alipour, & Khayatzadeh, 2015; Ma, Wang, Shen, Zhang, & Wu, 2014; Melero et al., 2012; Piccirillo, Dotto, Salata, & Giacomelli, 2013; Wieczorek, Denis, Lynch, & Osek, 2013). Some antimicrobials, such as quinolones and macrolides, have aroused the highest concern because of their significance in human medicine. Most cases of campylobacteriosis are usually self-limiting and do not require hospitalization. Antibiotic therapy is required, however, when severe enteritis or complications are present, as well as when very young or elderly patients and pregnant women are infected (Ge, Wang, Sjolund-Karlsson, & McDermott, 2013). In these cases, the macrolide erythromycin is usually the first-choice drug whereas fluoroquinolones and, to a less extent, tetracycline is alternative option (Allos, 2001). The indiscriminate use of antibiotics in animal husbandry and in the human population has leaded to an increase in antibiotic-resistant infections, which represents a hazard to the effectiveness of antimicrobial treatment. Thus, monitoring and obtaining information on *Campylobacter* resistance is highly relevant to public health and the international trade.

Among the resistance mechanisms of bacteria, integrons are now considered as efficient vehicles for the transfer of resistance markers among unrelated bacterial populations, especially those Gram-negative bacteria groups (van Essen-Zandbergen, Smith, Veldman, & Mevius, 2007). Integron structures are naturally occurring gene expression systems that can capture and integrate one or more gene cassettes and convert them into functionally expressed genes. It is these gene cassettes that encode the resistance determinants to several antimicrobial agents. To date, nine classes of integrons have been described and class 1 integrons are clinically significant (O'Halloran, Lucey, Cryan, Buckley, & Fanning, 2004). Integron-like structures were reported in *Campylobacter* isolates, suggesting that gene cassettes encoding antimicrobial resistance may act as a possible vehicle for the dissemination of resistance among *Campylobacter* spp (Ekkapobyotin, Padungtod, & Chuanchuen, 2008; Lee et al., 2002; van Essen-Zandbergen et al., 2007).

Horizontal transfer of gene cassettes together with any genetic modification of pre-existing genes through point mutation or some other genetic event, are thought to be the main mechanisms contributing to bacterial resistance (O'Halloran et al., 2004). For *Campylobacter* spp., most variability of resistance is found among fluoroquinolone, macrolides and tetracycline resistance, and it is important to examine the resistance determinants and their genetic composition as well as the resistance to antimicrobials. This might be very helpful to prevent the dissemination of resistance and for understanding the relationships in antimicrobial resistance between bacterial populations. In *Campylobacter* spp., the resistance to tetracycline is primarily mediated by a ribosomal protection protein (*tetO*), which is transferred as plasmid-encoded gene (Gibreel et al., 2004), or in the chromosome where it is not self-mobile (Chopra & Roberts, 2001). For quinolones and macrolides, resistance has mainly been associated with target modification in the quinolone resistance-determining region (QRDR) of the *gyrA* gene (Bakeli et al., 2008; Zirnstein, YU, Swaminathan, & Angulo, 1999) or mutations at positions 2074 and 2075 (domain V) in the *rrn* gene, which encodes for 23S rRNA (Alonso et al., 2005; Kurinčič, Botteldoorn, Herman, & Smole Možina, 2007).

Tracing the sources and understanding the epidemiology of *Campylobacter* infections is increasingly relying on molecular typing (Taboada et al., 2013). Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory technique which has been extensively used for the molecular typing of *Campylobacter* spp., mainly for *C. jejuni*. However, compared with *C. jejuni*, far less typing studies have been published on other *Campylobacter* spp., including *C. coli*. At the same time, important differences in exposure were identified with human *C. jejuni* and *C. coli* infections (Duarte et al., 2014; Ruiz-Castillo, Torres-Sánchez, & Aznar-Martín, 2014), suggesting that studies should be conducted at the species level.

Some recent studies have reported *Campylobacter* contamination in chicken in European countries (Economou et al., 2015; Guyard-Nicodème et al., 2015; Torralbo et al., 2015). However, from an extensive review of the international scientific literature, few reports were found on the occurrence and antimicrobial resistance of *Campylobacter* in chicken carcasses in China (Chen et al., 2010; Ma et al., 2014). Moreover, it is still less-known for the distribution of resistance determinants among *Campylobacter* isolates in China. While there were several studies describing the prevalence of *C. jejuni* and *C. coli* isolates from chickens, few have investigated the genotypes of the isolates from slaughter (Ma et al., 2014).

This study aims to determine the prevalence of *Campylobacter* spp. in the processing of broiler slaughter, examine the resistance to several antimicrobials and characterize the common mechanisms of resistance to fluoroquinolone, macrolide and tetracycline among the isolates. In addition, PFGE was performed to explore the diversity and links of *Campylobacter* species within the slaughter processing.

2. Materials and methods

2.1. Collection of samples

The investigation was conducted at one slaughterhouse from April 2012 to March 2013 in a vertically-integrated commercial poultry production continuum in Sichuan Province, China, in which more than 30 million broiler chickens were reared, slaughtered and sold per year. A total of 451 broiler cecal samples (one from each

broiler), representing samples of broiler at the farm level, were collected after evisceration. One hundred samples were collected by aseptically swabbing on the surface of the carcasses at the evisceration stage, and one hundred samples were sampled by swabbing on the surface of the carcass parts at the cut-up stage. Then the swabs were immediately put into 3 ml of selective Bolton broth containing 5% of fresh sterile defidrinated sheep blood. All of the samples were transported to the laboratory on ice within 3 h of collection and were analyzed as soon as possible.

2.2. Isolation and identification of Campylobacter

For each cecal sample or selective enrichment broth, a loopful of fecal material was directly streaked onto modified Skirrow agar plate (Sigma, St. Louis, MO, USA) containing 5% fresh sterile sheep blood and *Campylobacter* supplement III (Sigma), and then incubated at 42 °C in a microaerophilic (5%O_2, 10%CO_2 and 85%N_2) chamber for 48–72 h. One presumptive *Campylobacter* colony from each selective agar plate was subcultured and identification was performed using standard microbiological and biochemical procedures including Gram staining, production of catalase, oxidase according to ISO 10272-1:2006. The bacteria isolates that showed typical growth on mCCDA, were gram negative, had corkscrew-like darting motility, were oxidase positive and did not show growth at 41.5 ± 0.5 °C in aerobic conditions or at 25 °C in microaerobic conditions, were considered as *Campylobacter* spp.

2.3. DNA extraction and multiplex PCR identification

For each positive plate, up to three presumptive *Campylobacter* colonies were selected for further identification using multiplex PCR (m-PCR). *Campylobacter* chromosomal DNA was extracted using a MiniBEST Bacteria Genomic DNA Extraction Kit (Takara, Japan). PCR were conducted with three sets of primers (Table 1) specific for the simultaneous detection of the *C. jejuni* (the *hipO* gene target), *C. coli* (*ceuE* gene) and *Campylobacter*-specific 16S rRNA gene (positive control). The primers for identification of *hipO* gene in this work were designed by Oligo 6.0 software. PCR was performed in a 25 μL mixture containing 2 × PCR Mix 12.5 μL (Takara), DNA template 1.0 μL and a final concentration of 0.8 μmol/L 16S rRNA primers, 0.4 μmol/L *hipO* primers and 0.2 μmol/L *ceuE* primers. Amplification reactions were carried out using a DNA thermal cycler (C1000, Bio-rad, USA) with the following program: one cycle of 10 min at 95 °C, 30 cycles each consisting of 30 s at 95 °C, 1 min and 30 s at 56 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. Reference strains of *C. jejuni* (ATCC 33560) and *C. coli* (ATCC 33559) were used as positive controls and sterile distilled water was used as no template control (NTC). The PCR products were stained with 1% ethidium bromide and visualized under UV light after gel electrophoresis on 1.5% agarose gel. Where isolates from the same sample, only one of the isolates that belonged to the same species was selected for the subsequent analysis. All confirmed isolates were stored at −80 °C in Brain Heart Infusion (BHI) broth (Hangwei, Hangzhou, China) containing 20% glycerol.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by a standard agar dilution method in Muller-Hinton agar supplemented with 5% sheep blood, as described by the Clinical and Laboratory Standards Institute (CLSI, 2010). The agar plates were incubated at 42 °C for 24 h under a microaerophilic atmosphere. The following antimicrobial agents were used: levofloxacin (LEV), ciprofloxacin (CIP), streptomycin (STR), gentamicin (GEN), florfenicol (FLO), tetracycline (TET), clindamycin (CLI) and erythromycin (EM). All the antimicrobial agents were obtained from China Institute of Veterinary Drug Control (Beijing, China). *C. jejuni* ATCC33560 was used as the quality control organism.

The breakpoint value for erythromycin, ciprofloxacin, and tetracycline used in this study was determined as ≥32 μg/mL, 4 μg/mL and 16 μg/mL, respectively, according to Clinical and Laboratory Standards Institute (CLSI, 2010). For clindamycin, the susceptibility breakpoint (MIC, ≥8 mg/L) of the National Antimicrobial Resistance Monitoring System (NARMS) were used (http://www.cdc.gov/narms/). Since there is no breakpoint criteria for gentamicin and levofloxacin for Campylobacter according to CLSI, the breakpoint value for gentamicin and levofloxacin used in this study was that the MIC ≥ 16 μg/mL and 8 μg/mL for *Enterobacteriaceae* (CLSI, 2012). The breakpoint value for streptomycin and florfenicol were set as MIC ≥ 16 μg/mL and 32 μg/mL according to previous publications (Sahin et al., 2008; Zhang et al., 2014). Multidrug resistance was defined as resistance to three or more (≥3) classes of antimicrobials.

2.5. Integron analysis

A random collection (n = 146) of MDR Campylobacter isolates were investigated for the presence of class 1 integrons, genetic elements associated with the dissemination of antimicrobial resistance by PCR as described before (Table 2) (Ekkapobyotin et al., 2008). Each isolate was analyzed for the presence of gene cassettes associated with class 1 integron structures using a PCR assay described by the reference with modifications (Lévesque et al., 2005). Generally, PCR assays were carried out in a final volume of 25 μL using 2 × long Taq PCR MasterMix kit (Takara, Japan) according to the manufacturer's instructions in a C1000 Thermal Cycler (Bio-Rad, USA).

Specific amplicons of gene cassettes fragments (n = 20) were gel purified using QIAQuick Gel Extraction kit (Qiagen, Germany) and submitted for DNA sequencing (Takara, Japan). Then the resulting DNA sequences were compared with the GenBank Database using the Blast algorithm available at the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov).

2.6. Detection of tetO gene, analysis of mutations in 23S rRNA and QRDRs of gyrA gene

PCR amplification of *tetO* gene, 23S rRNA and the QRDRs of *gyrA* gene were performed using the primers listed in Table 2. For the amplification of *tetO* gene, the PCR mixture was subjected to the

Table 1
PCR primers used for identification of *Campylobacter* spp.

Organism	Primer	Sequence (5′→3′)	Amplicon length (bp)	Reference
Campylobacter spp.	16S rRNA	F: TCTAATGGCTTAACCATTAAAC R: GGACGGTAACTAGTTTAGTATT	857	(Denis et al., 1999)
C. jejuni	*hipO*	F: TGATGGCTTCTTCGGATAG R: CTAGCTTCGCATAATAACTTG	600	This study
C. coli	*ceuE*	F: ATTGAAAATTGCTCCAACTATG R: GATTTTATTATTTGTAGCAGCG	462	(Denis et al., 1999)

Table 2
Target genes, primers and annealing temperatures used for detection of integron, linked gene cassettes and resistance determinant genes.

Target gene	Primer sequences (5′-3′)	Temperature (°C)	Amplicon size (bp)	Reference
Integron 1	*Int1*L-F: CAGGAGATCGGA AGACCT *Int1*L-R: TTGCAA ACCCTCACTGAT	60	150	(Ekkapobyotin et al., 2008)
Gene cassette	CS-F: GGCATCCAAGCAGCAAGC CS-R: AAGCAGACTTGACCTGAT	55	Varied length	(Lévesque et al., 2005)
tetO	*tetO*-F: GGCGTTTTGTTTATGTGCG *tetO*-R: ATGGACAACCCGACAGAAGC	55	559	(Gibreel et al., 2004)
23S rRNA	23SRNA-F: TTAGCTAATGTTGCCCGTACCG 23SRNA-R: AGCCAACCTTTGTAAGCCTCCG ERY2075-R: TAGTAAAGGTCCACGGGGTCGC	50	697 485	(Alonso et al., 2005)
gyrA	*gyrA*-F1: TTTTTAGCAAAGATTCTGAT *gyrA*-R1: CAAAGCATCATAAACTGCAA *gyrA*-R2: CAGTATAACGCATCGCAG	49	265 368	(Zirnstein et al., 1999)

following thermal cycles: one cycle at 95 °C for 5 min; 30 cycles of 95 °C for 45s, 55 °C for 45s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 5min. For amplification of 23S rRNA, the PCR conditions were according to the reference (Alonso et al., 2005) with modifications. The conserved forward primer 23SRNA-F was used in conjunction with the reverse mutation primer ERY2075 to detect A2075G mutation. In each reaction, an amplicon of 485bp could be expected, when the isolate carried the corresponding mutation. The annealing and extension conditions were changed to 55 °C for 30s and 72 °C for 45s. Meanwhile, a fragment from 23S rRNA gene of *C. jejuni* and *C. coli* was used in this assay to generate a 697 bp amplicon for DNA sequencing. Finally, the amplified PCR products were visualized by electrophoresis in a 2% agarose gel stained with ethidium bromide (2 μg/mL). In order to confirm the mutation associated with resistance, the PCR products of the 23S rRNA were analyzed by DNA sequencing (Takara, Japan). Two *C. coli* isolates and two *C. jejuni* susceptible to erythromycin were included as controls.

The detection of QRDR mutation was performed using mismatched amplification mutation assay (MAMA) PCR described by previous study (Zirnstein et al., 1999) with modifications. The PCR system was as following, a conserved forward primer (*gyrA*F1) was used in conjunction with the mutation primer (*gyrA*R1) to generate a 265 bp PCR product that was a positive indication of the presence of the Thr-86-Ile (ACA→ATA) mutation in the *gyrA* gene, and in parallel with a conserved reverse primer (*gyrA*R2) to produce a positive PCR control product of 368 bp with any *Campylobacter gyrA* gene. PCR conditions were as follows: initial denaturation was at 95 °C for 30s, 49 °C for 30s, and 72 °C for 30s. Ten-microliter aliquots of each product were loaded onto horizontal, 2.0%, 0.5 Tris-borate-EDTA agarose gels and were stained with ethidium bromide for analysis after electrophoresis. Furthermore, the 265bp-sized DNA amplicons (n = 10) were submitted for DNA sequencing (Takara, Japan) and the resulting DNA sequence data were compared with the published DNA sequences of *C. jejuni* and *C. coli* for *gyrA* corresponding area. Two *C. coli* isolates and two *C. jejuni* susceptible to ciprofloxacin were included as controls.

2.7. PFGE typing

Pulsed-field gel electrophoresis (PFGE) was performed to generate genomic DNA fingerprinting profiles of *C. jejuni* and *C. coli* isolates using *Sma*I (Takara, Japan) according to the procedures developed by the Centers for Diseases Control and Prevention (CDC, http://www.cdc.gov/pulsenet/pathogens/campylobacter.html). Meanwhile, *Salmonella* H9812 as the reference marker (digested with *Xba*I). Agarose embedded DNA was digested for 2 h at 25 °C with 40 U of *Sma*I. Resulting restriction fragments of *Campylobacter* isolates were separated by PFGE in 1% agarose gels with 0.5 × TBE buffer. Electrophoresis was performed in a CHEF-DR III system (Bio-Rad Laboratories), with initial switch time of 6.76 s; final switch time of 38.35 s; 120°angle; 6.0 V/cm gradient; at 14 °C; running for 19 h. After electrophoresis, gels were stained in a 40 μg/mL solution of ethidium bromide for 30 min and then washed with distilled water. Gels' images were visualized and captured with a UV transilluminator (Gel Doc XR, Bio-Rad).

2.8. Statistical analysis

PFGE results were analyzed using the BioNumerics software version 3.0 (Applied Maths), the banding patterns were clustered using Dice coefficients with a 1.0% band position tolerance and 1.0% optimization. The unweighted pair-group method using arithmetic averages (UPGMA) was used to cluster patterns. Isolates with less than 85% similarity according to the dendrogram were clustered as separate genotypes.

3. Results

3.1. Occurrence of Campylobacter species

Out of 651 samples from slaughter processing, 333 (51.2%) were presumed as positive for *Campylobacter* spp. according to the phenotype tests. The isolates were further confirmed by m-PCR and the results indicated that *C. jejuni* was the predominant species (24.6%, n = 160), followed by *C. coli* (20.0%, n = 130). Eleven isolates could not be differentiated further. A total of 253 Campylobacter isolates were obtained from cecal samples, including 156 were positive for *C. jejuni* and 87 for *C. coli*. In addition, there were 12 cecal samples which were simultaneously positive for both *C. jejuni* and *C. coli*. For the 100 samples from carcasses surface after evisceration, 31 (31%) samples were positive for Campylobacter spp., among which 4 and 26 were identified as *C. jejuni* or *C. coli*, respectively (Table 3). Among 100 samples from carcass parts, 17 (17%) were identified as positive for *C. coli*, none was identified as positive for *C. jejuni*.

3.2. Antimicrobial susceptibility

The results of antimicrobial susceptibility testing performed on the 160 *C. jejuni* and 130 *C. coli* isolates are presented in Table 4. For *C. jejuni*, resistance to CIP was the most common finding (88.1%), followed by resistance to TET (79.4%) and LEV (78.1%), to a less extent to FLO (16.3%). As for *C. coli*, most of the isolates were resistant to CIP (100%), followed by resistance to TET (98.5%), LEV (98.5%), CLI (98.5%) and EM (93.9%); few strains were resistant to STR (17.8%) and FLO (8.5%).

Overall, all of 290 *Campylobacter* isolates (100%) were resistant

Table 3
Occurrence of *Campylobacter* species isolated from ceca, carcasses and carcass parts in the slaughterhouse.

Sources of samples	No. (%) of isolates			
	C. jejuni	*C. coli*	*C. jejuni* + *C. coli*	*Campylobacter* positive no.
Cecal samples	34.6% (156/451)	19.3% (87/451)	2.7% (12/451)	56.1% (253/451)
Carcasses	4.0% (4/100)	26.0% (26/100)	0.4% (2/451)	31.0% (31/100)
Carcass parts	0 (0/100)	17.0% (17/100)	0 (0%100)	17.0% (17/100)
Total	24.6% (160/651)	20.0% (130/651)	3.1% (14/451)	

to one or more antimicrobial agent. As many as two isolates (0.7%) were resistant to only one antimicrobial agent tested and 16 isolates (5.5%) showed resistance to two antimicrobial agents. Multidrug resistance which was defined as resistance to three or more drugs was found in 272 strains (93.8%) of *Campylobacter* isolates (Table 5). Moreover, 91.3% (146/160) of the *C. jejuni* isolates were MDR strains, which were mainly resistant to 3–6 kinds of antimicrobial agents (12.64%, 13.95%, 97% and 9.30%, respectively). By contrast, MDR strains occupied 99.2% (129/130) of the *C. coli* isolates, which mainly showed resistance to 6 (32.23%) or 7 kinds (7.97%) of drugs.

3.3. Class 1 integron and relevant gene cassettes for Campylobacter spp. isolates

Totally 146 MDR *Campylobacter* isolates were screened for the presence of class 1 integrons. As a result, class 1 integrons were found in 144 isolates (98.6%) among which 98.7% (77/78) were positive for *C. jejuni* and 98.5% (67/68) for *C. coli* isolates. Additionally, all the *intI1*-positive strains were assayed for the presence of gene cassettes linked to class 1 integrons. The results indicated that 3 kinds of gene cassettes-associated amplicons were generated. Consequently, 72 isolates produced amplicons of 1000–750–500–250 bp, which was the predominant pattern. Besides, 3 isolates produced amplicons of 1000–750 bp, and 3 isolates produced an amplicon of 1000-bp (Table 6). Since the average size of a bacterial coding sequence is at least 800 bp, amplicons of 1.0 kb from 20 randomly selected isolates were pursued with further investigation, on the basis that these were more likely to contain a complete ORF corresponding to a potential gene. As a result, nucleotide sequencing and BLAST searches with larger ORF identified it as an *aadA2*-encoding aminoglycoside adenyltransferase associated with the aminoglycoside resistance.

3.4. Occurrence of tetO gene

In the screening of tetracycline resistance genes among the isolates, 94.6% (123/130) of tetracycline resistant isolates (MIC $\geq$ 16) were positive for the carriage of *tetO* gene, but none of tetracycline-susceptible isolates did. The possession of *tetO* gene was found in 96.6% (57/59) of *C. jejuni* and 94.1% (64/68) of *C. coli* isolates, respectively.

Table 4
Prevalence of antimicrobial resistance in *Campylobacter* isolates from chicken slaughtering process.

Antimicrobial agents	Antimicrobial resistance rates of tested Campylobacter isolates % (n)[a]	
	C. jejuni % (N = 160)[b]	*C. coli* % (N = 130)[b]
LEV	78.1% (125)	98.5% (128)
CIP	88.1 (141)	100% (130)
STR	43.8% (70)	17.8% (23)
GEN	47.5% (76)	90.8% (118)
FLO	16.3% (26)	8.5% (11)
TET	79.4% (127)	98.5% (128)
CLI	68.1% (109)	98.5% (128)
EM	14.3% (23)	93.9% (122)

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

3.5. Mutations within 23S rRNA

The conserved forward primer 23SRNA-F was used in conjunction with the reverse mutation primer ERY2075-R to detect the A2075G mutation. An amplicon of 485 bp could be expected, when the isolates carried the corresponding mutation. Otherwise, the amplicon would not be acquired in wild-type strains because the last base in the 3′ end of 23SRNA-R primer was not matching efficiently with the corresponding position of wild-type strains. PCR amplification and nucleotide sequences analysis of 23S rRNA was performed in erythromycin-resistant isolates to detect mutations associated with resistance to macrolides, and the results indicated that 96.4% of erythromycin-resistant strains possessed A2075G mutation in the V domain of 23S rRNA sequences. The presences of this mutation were detected in 95.0% of *C. jejuni* and in 96.7% of *C. coli*, respectively. No such mutation was observed in 23S rRNA of the erythromycin-sensitive control.

3.6. Mutations within the QRDR of gyrA gene

An examination of the *gyrA* DNA mutation in QRDR using MAMA PCR is convenient for the determination of the presence or absence of amino acid mutations in the QRDR. Isolates with the wild-type amino acid 86 codon (ACA, ciprofloxacin susceptible) were not amplified with the reverse mutation primer *gyrA*R1, whereas the isolates with the mutated amino acid 86 codon (ATA, ciprofloxacin resistant) generated a 265-bp PCR product with the *gyrA*R1 reverse mutation primer and the *gyrA*F1 forward conserved primer (Table 3). Conserved primers *gyrA*F1 and *gyrA*R2 generated a 368-bp *gyrA* PCR product with DNA isolated from all of the isolates in this study. Replacement of C256 with T leading to a Thr-86-Ile substitution in *gyrA* was the most common mutation, 93.1% (135/145) of ciprofloxacin-resistant *Campylobacter* isolates had a substitution at amino acid position 86 of the *gyrA* protein due to a mutation of the DNA codon from ACA (threonine) to ATA (isoleucine). Totally, 97.3% (71/73) of *C. jejuni* isolates and 89.7% (61/68) of *C. coli* isolates possessed a Thr-86-Ile substitution in *gyrA*, however mutation of Thr-86-Ile was not found in ciprofloxacin sensitive Campylobcter strains. From DNA sequences analysis, 2 point mutations in *gyrA* were also identified in some isolates, which i.e. T-243-C and T-320-C were silent mutations.

3.7. PFGE patterns of Campylobacter isolates

A total of 78 *C. jejuni* and 68 *C. coli* isolates, representing isolates of different origins, species, resistance patterns and resistance determinants were selected for PFGE analysis after digestion by *Sma*I.

As a result, 33 PFGE profiles were generated among 78 isolates of *C. jejuni*. The patterns consisted of 6–16 distinct fragments

Table 5
Antimicrobial resistant profiles of *Campylobacter jejuni* and *C. coli* from chicken slaughtering process.

No. of antimicrobials (Classes)	Resistance patterns	Cecal samples (n = 243)	Carcasses (n = 30)	Carcass parts (n = 17)
1 (1)	LEV	2		
2 (2)	TET CLI	4		
2 (3)	STR TET	2		
2 (4)	CIP CLI	1		
2 (5)	CIP GEN	4		
2 (6)	CIP LEV	5		
3 (7)	STR CLI EM	1		
3 (8)	GEN TET CLI	1		
3 (9)	LEV TET CLI	1		
3 (10)	LEV GEN STR	1		
3 (11)	CIP CLI EM	1		
3 (12)	CIP TET CLI	2		
3 (13)	CIP STR TET	1		
3 (14)	CIP GEN CLI	3		
3 (15)	CIP GEN STR	5		
3 (16)	CIP LEV CLI	1		
3 (17)	CIP LEV TET	21		
3 (18)	CIP LEV GEN	1		
4 (19)	GEN STR TET EM	1		
4 (20)	GEN STR TET CLI	1		
4 (21)	LEV TET CLI EM	1		
4 (22)	LEV GEN STR CLI	2		
4 (23)	LEV GEN STR TET	2		
4 (24)	CIP GEN STR CLI	1		
4 (25)	CIP GEN STR TET	4		
4 (26)	CIP LEV TET FLO	2		
4 (27)	CIP LEV TET CLI	23	1	
4 (28)	CIP LEV STR CLI	1		
4 (29)	CIP LEV GEN CLI	1		
4 (30)	CIP LEV GEN TET	1		
4 (31)	CIP LEV GEN STR	5		
5 (32)	LEV GEN STR TET CLI	1		
5 (33)	CIP GEN TET CLI EM	1		
5 (34)	CIP GEN STR TET CLI	1		
5 (35)	CIP LEV TET CLI FLO	7	3	
5 (36)	CIP LEV TET CLI EM	4		
5 (37)	CIP LEV STR TET CLI	6		
5 (38)	CIP LEV GEN TET CLI	3		
5 (39)	CIP LEV GEN STR CLI	1		
5 (40)	CIP LEV GEN STR TET	2		
6 (41)	CIP LEV TET CLI EM FLO	1	2	
6 (42)	CIP GEN STR TET CLI FLO	1		
6 (43)	CIP LEV STR TET CLI FLO	3	1	
6 (44)	CIP LEV GEN TET CLI EM	62	19	14
6 (45)	CIP LEV GEN STR TET EM	1		
6 (46)	CIP LEV GEN STR TET CLI	14		
6 (47)	CIP GEN STR TET CLI EM	1		
7 (48)	LEV GEN STR TET CLI EM FLO	1		
7 (49)	CIP LEV GEN STR TET CLI FLO	7		
7 (50)	CIP LEV GEN TET CLI EM FLO	1	1	3
7 (51)	CIP LEV GEN STR TET CLI EM	19	1	
8(52)	CIP LEV GEN STR TET CLI EM FLO	4	2	

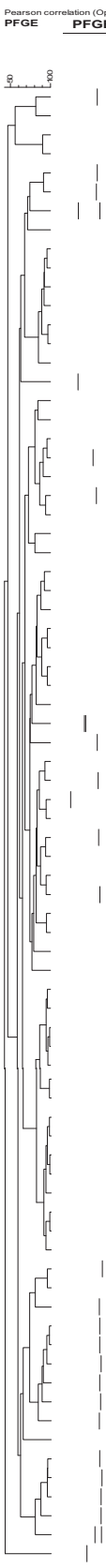
Table 6
Patterns of gene cassettes of Campylobacter isolates from chicken slaughtering process.

Gene cassettes pattern	Amplicons	Isolates types and no. (n)	Isolates origin and no. (n)
Pattern I	1000 bp	*C. jejuni* (3)	JF (3)
PatternII	1000–750 bp	*C. jejuni* (3)	JF (3)
Pattern III	1000–750–500–250 bp	*C. jejuni* (7), *C. coli* (65)	JF (27), JH (28), JR (17)

JF, cecal samples; JH, carcasses after evisceration; JR, carcasses after dissection. n, number of Campylobacter isolates.

ranging in size from less than 20.5 kb–668.9 kb after *Sma*I digestion. The dendrogram, obtained from numerical analysis of macrorestriction patterns of *C. jejuni* isolates, is shown in Fig. 1. The similarity level of the *C. jejuni* isolates varied from 42.1% to 99.1%. We could identify three large clusters at the similarity level above 50%. The first cluster contained pattern 1 to 3 with 4 isolates, which shared a similarity level of 56.1%. The second cluster was identified with pattern 4 to 32 including 73 isolates, which occupied 93.6% of the isolates; the similarity level among them was 58.4%. Lastly, the third cluster was pattern 33 with only one isolates.

The *C. jejuni* isolates were grouped into 19 unique PFGE patterns (PFGE patterns represented by a single strain) and 14 clusters (PFGE pattern represented by multiple strains). Among 78 *C. jejuni* isolates available, 74 isolates were recovered from cecal samples, only 4



Strains	Genotypes	AMR patterns	Resistance determinants				
			A	B	C	D	E
13-JF74	1 JF-CJ	CIP LEV GEN TET CLI EM	+	-	+	-	+
12-JF20	2 JF-CJ	LEV GEN STR TET CLI EM FLO	+	-	-	+	+
12-JF109	3 JF-CJ	CIP LEV STR TET CLI FLO	+	-	+	+	-
12-JF59	3 JF-CJ	CIP LEV TET CLI EM	+	-	+	+	+
13-JF65	4 JF-CJ	CIP LEV TET CLI	+	-	+	+	+
13-JF63	4 JF-CJ	CIP LEV TET CLI EM FLO	+	-	+	+	+
12-JF79	5 JF-CJ	CIP LEV TET CLI FLO	+	-	+	+	+
JF-74	6 JF-CJ	CIP LEV STR TET CLI	+	aadA2	+	+	+
12-JF17	7 JF-CJ	CIP LEV TET CLI EM	+	aadA2	+	+	+
12-JF26	7 JF-CJ	CIP LEV TET FLO	+	aadA2	+	+	+
12-JF52	7 JF-CJ	CIP TET CLI	+	aadA2	+	+	+
12-JF148	7 JF-CJ	CIP LEV GEN TET CLI	+	-	+	+	+
12-JF174	7 JF-CJ	CIP GEN STR CLI	+	-	+	+	+
12-JF138	7 JF-CJ	LEV TET CLI EM	+	-	+	+	+
12-JF122	7 JF-CJ	CIP LEV TET	+	-	+	+	+
12-JF152	8 JF-CJ	CIP LEV GEN STR TET CLI	+	-	+	+	+
12-JF68	9 JF-CJ	CIP LEV STR TET CLI FLO	+	-	+	+	+
12-JF164	10 JF-CJ	CIP LEV GEN STR TET	+	-	+	+	+
13-JF64	11 JF-CJ	CIP LEV GEN STR TET CLI EM FLO	+	-	+	+	+
12-JF108	11 JF-CJ	CIP LEV STR TET CLI	+	-	+	+	+
13-JF93	11 JF-CJ	CIP LEV GEN TET CLI EM	+	-	+	+	+
12-JF168	11 JF-CJ	CIP STR TET	+	-	+	+	+
12-JF150	11 JF-CJ	CIP LEV TET CLI	+	-	+	+	+
12-JF65	12 JF-CJ	CIP LEV STR TET CLI FLO	+	-	+	+	+
12-JF153	13 JF-CJ	CIP LEV GEN CLI	+	-	+	+	+
13-JF61	14 JF-CJ	CIP LEV GEN STR TET CLI EM FLO	+	-	+	+	+
12-JF22	14 JF-CJ	CIP LEV GEN STR TET CLI FLO	+	aadA2	+	+	+
13-JF69	14 JF-CJ	CIP LEV GEN STR TET CLI EM	+	-	+	+	+
12-JF165	15 JF-CJ	GEN STR TET EM	+	-	+	+	+
1-14	15 JH-CJ	CIP LEV STR TET CLI FLO	+	aadA2	+	+	+
12-JF163	15 JF-CJ	CIP LEV TET	+	-	+	+	+
12-JF156	15 JF-CJ	CIP LEV EM	+	-	+	+	+
12-JF119	16 JF-CJ	CIP LEV GEN STR TET CLI FLO	+	-	+	+	+
12-JF185	17 JF-CJ	CIP LEV GEN STR TET CLI	-	-	+	+	+
13-JF80	18 JF-CJ	CIP LEV GEN STR TET CLI EM	+	-	+	+	+
1-9	19 JH-CJ	CIP LEV TET CLI FLO	+	-	+	+	-
1-11	19 JH-CJ	CIP LEV GEN STR TET CLI EM FLO	+	-	+	+	+
12-JF16	19 JF-CJ	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
12-JF183	19 JF-CJ	CIP LEV GEN STR TET EM	+	-	+	+	+
12-JF161	20 JF-CJ	CIP GEN CLI	+	-	+	+	+
12-JF187	20 JF-CJ	CIP GEN CLI	+	-	+	+	+
12-JF126	20 JF-CJ	GEN STR TET CLI	+	-	+	+	+
12-JF154	20 JF-CJ	CIP GEN CLI	+	-	+	-	-
12-JF135	21 JF-CJ	LEV GEN STR CLI	+	-	+	+	+
12-JF110	21 JF-CJ	CIP LEV STR CLI	+	-	+	+	+
12-JF134	22 JF-CJ	CIP GEN STR	+	-	+	+	+
12-JF136	23 JF-CJ	LEV GEN STR TET	+	-	+	+	+
12-JF175	24 JF-CJ	CIP LEV TET	+	-	+	+	-
12-JF189	24 JF-CJ	CIP LEV GEN STR TET CLI	+	-	+	+	+
12-JF177	24 JF-CJ	CIP GEN STR TET CLI	+	-	+	+	+
13-JF62	24 JF-CJ	CIP LEV GEN STR TET CLI EM FLO	+	-	+	+	+
12-JF129	24 JF-CJ	CIP LEV GEN	+	-	+	+	+
12-JF128	24 JF-CJ	CIP LEV GEN STR CLI	+	-	+	+	+
12-JF143	24 JF-CJ	CIP LEV GEN STR TET CLI FLO	+	-	+	+	+
12-JF178	25 JF-CJ	CIP GEN STR TET CLI FLO	+	-	+	+	+
12-JF186	25 JF-CJ	CIP LEV GEN STR TET CLI	+	-	+	+	+
12-JF182	25 JF-CJ	CIP GEN STR	+	-	+	+	+
12-JF181	25 JF-CJ	CIP LEV GEN STR	+	-	+	+	+
12-JF139	25 JF-CJ	LEV GEN STR	+	-	+	+	+
12-JF53	25 JF-CJ	CIP LEV TET CLI EM	+	aadA2	+	+	+
12-JF137	25 JF-CJ	LEV GEN STR CLI	+	-	+	+	+
12-JF142	25 JF-CJ	CIP LEV GEN STR TET	+	-	+	+	+
12-JF112	26 JF-CJ	CIP LEV CLI	+	-	+	+	+
13-JF60	26 JF-CJ	----	+	-	-	-	-
12-JF21	27 JF-CJ	CIP LEV TET FLO	+	aadA2	+	+	+
12-JF170	28 JF-CJ	CIP GEN STR TET	+	-	+	+	+
1-5	28 JH-CJ	CIP LEV TET CLI FLO	+	aadA2	+	+	+
12-JF97	28 JF-CJ	STR CLI EM	+	-	-	+	+
12-JF123	28 JF-CJ	CIP LEV TET CLI FLO	+	-	+	+	+
12-JF125	28 JF-CJ	GEN TET CLI	+	-	+	+	+
12-JF57	29 JF-CJ	CIP TET CLI	+	aadA2	+	+	+
12-JF176	30 JF-CJ	CIP GEN TET CLI EM	+	-	+	+	-
12-JF7	31 JF-CJ	CIP LEV TET CLI	+	aadA2	+	+	+
12-JF140	31 JF-CJ	CIP LEV GEN STR	+	-	+	+	+
12-JF132	31 JF-CJ	CIP LEV GEN TET	+	-	+	+	+
12-JF169	31 JF-CJ	LEV GEN STR TET	+	-	+	+	+
12-JF2	32 JF-CJ	CIP LEV TET CLI	+	-	+	-	+
12-JF19	33 JF-CJ	LEV TET CLI	+	aadA2	-	+	+

Fig. 1. Dendrogram of *Sma*I PFGE types, antimicrobial resistance (AMR) patterns and determinant mutations of *C. jejuni* isolates recovered from cecal samples, carcasses at the slaughterhouse. 1–33, PFGE patterns. JF, cecal samples; JH, carcasses. A, integron1; B, gene cassettes; C, *gyrA*; D, *tetO*; E, 23S rRNA. +, positive; –, negative. CIP, ciprofloxacin; STR, streptomycin; GEN, gentamicin; FLO, florfenicol; TET, tetracycline; CLI, clindamycin; EM, erythromycin; LEV, levofloxacin.

isolates were from carcasses. High genotype diversity was presented among 74 isolates of *C. jejuni* from cecal samples, which revealed that the isolates were from a variety of flocks. On the other hand, the isolates with identical genotypes may originate from different sources, such as strains of 12-JF165 and 1–14 in pattern 15, isolates in pattern 19 and 28, which indicated that strains may circulate from feces to the carcasses or environment.

The most common one, pattern 25-CJ, was highlighted for 8 isolates showing different antimicrobial resistant types. Besides, the 24-CJ PFGE profile (7 isolates) was very similar to the first one (80.1% of similarity). Moreover, the third PFGE profile (7-CJ) shared by 7 isolates was clearly different from the two others. The analysis of the relatedness of the *C. jejuni* strains showed a poor correlation among PFGE profiles, antimicrobial resistance and determinant patterns. Generally, isolates with the same genetic resistance patterns belonged to different PFGE types and resistance patterns. On the other hand, 2 strains of *C. jejuni* (12-JF161 and 12-JF187) with identical genetic profile displayed the same PFGE type and antimicrobial resistance profile.

In contrast to *C. jejuni*, 15 PFGE patterns were produced among 68 *C. coli* isolates sharing a similarity level of 54.0%–99.7%, and each isolate yielded 7 to 14 distinct restriction fragments which varied from 20.5 kb to 668.9 kb in size after *Sma*I digestion (Fig. 2). The *C. coli* isolates were subdivided into 8 unique PFGE patterns and 7 clusters. Twenty six isolates from cecal samples generated 12 PFGE patterns, 25 isolates from carcasses produced 8 PFGE types and 17 isolates from carcass parts were subdivided into 5 genotypes. It seemed that the *C. coli* isolates were more closely related than the *C. jejuni* isolates.

PFGE pattern of 4-CC dominated the profiles, which occupied 44.1% of the isolates, including 30 isolates recovered from cecal samples (13 isolates), carcasses (12 isolates) and carcass parts (5 isolates). The majority of the isolates in pattern 4-CC showed an identical resistance type. The second predominant profile was pattern 11-CC, which accounted for 12 isolates recovered from cecal samples (1 isolates), carcasses (3 isolates) and carcass parts (8 isolates). All the isolates clustered in pattern 11 showed the same resistance type and resistant determinants.

Compared with *C. jejuni*, there is a number of *C. coli* isolates showing good relatedness among the PFGE pattern, the resistance type and determinants, such as PFGE pattern 4-CC, 6-CC, 8-CC and 11-CC, which showed that *C. coli* isolates belonging to the same genotype could be recovered from different origins. These results may suggest that a *C. coli* clone circulates in the slaughter processing, or that the *Sma*I is not discriminative enough for this strain.

4. Discussion

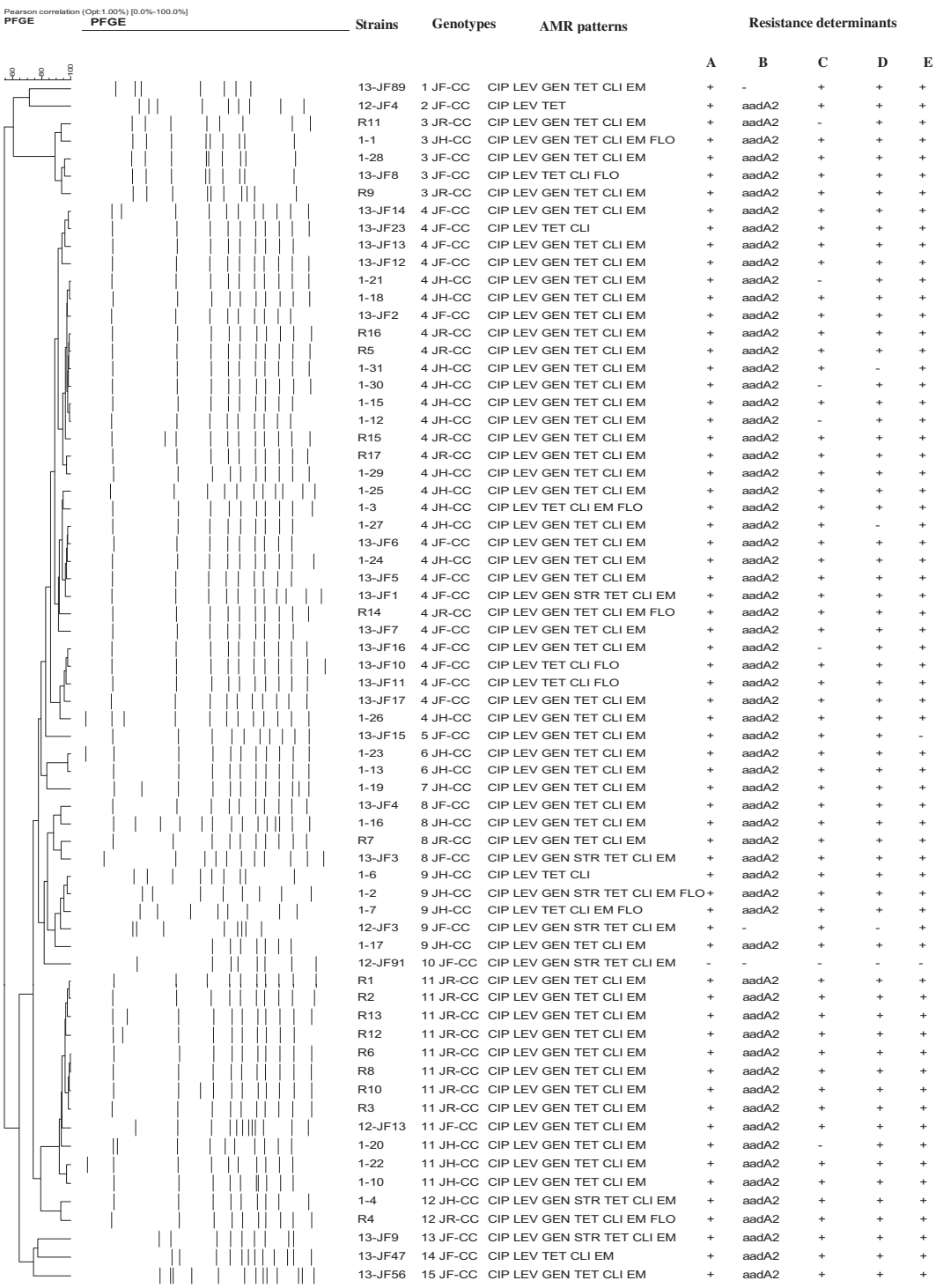
In the present study, the higher prevalence of *C. jejuni* in broiler chickens is contrary to the earlier findings conducted in Shanghai of China (Ma et al., 2014) and many reports from other countries including Reunion Island, Grenada and Spain (Hariharan, Sharma, Chikweto, Matthew, & DeAllie, 2009; Henry, Reichardt, Denis, & Cardinale, 2011; Torralbo et al., 2015), where *C. coli* was reported to be the predominant *Campylobacter* species in broiler chickens.

It was also found that *Campylobacter* strains were most commonly resistant to ciprofloxacin (88.1% for *C. jejuni* and 100% for *C. coli*), tetracycline (79.4% for *C. jejuni* and 98.5% for *C. coli*) and to a less extent to levofloxacin (78.1% for *C. jejuni* and 98.5% for *C. coli*). There was an obvious difference in the resistance to erythromycin between *C. jejuni* (14.3%) and *C. coli* (93.9%). Recent susceptibility studies on *Campylobacter* from poultry performed in different countries indicate substantial variation among countries. Relatively high resistance rates in *Campylobacter* isolates from chicken were reported from Spain (Torralbo et al., 2015) and Iran (Zendehbad, Khayatzadeh, & Alipour, 2015); moderate rates were reported from Greece (Economou et al., 2015) and Poland (Andrzejewska, Szczepańska, Śpica, & Klawe, 2015), whereas limited occurrence of antimicrobial resistance among was reported from France (Guyard-Nicodème et al., 2015) and Grenada (Hariharan et al., 2009). These differences on occurrences of antimicrobial resistance maybe reflect the different national and regional policies on the use of antimicrobial agents for food animals. Regarding China, fluoroquinolones, tetracyclines, macrolides and aminoglycosides are widely adopted to treat and prevent bacterial diseases in poultry, and the extensive and indiscriminate use of antimicrobials in broiler production has resulted in the high prevalence of antimicrobial resistance among *Campylobacter* species, which imposed a selective pressure on the emergence and spread of resistant *Campylobacter* isolates.

The 290 *Campylobacter* strains, among which all but two were resistant to two or more antimicrobial agents, exhibited 52 kinds of resistance patterns. Moreover, 272 of the 290 (93.8%) *Campylobacter* isolates exhibited multi-drug resistance, higher than figures reported from other countries, including Poland (Andrzejewska et al., 2015), Iran (Dallal et al., 2010), Ethiopia (Kassa, Gebre-Selassie, & Asrat, 2007) and France (Payot, Dridi, Laroche, Federighi, & Magras, 2004). The majority (32.8%, 95/290) of the strains were attributed to the resistance pattern of CIP-LEV-GEN-TET-CLI-EM and to a lesser extent of CIP-LEV-TET-CLI (8.3%, 24/290). Considering by species, multidrug resistance was more prevalent among *C. coli* (99.2%) than *C. jejuni* (91.3%), consistent with results reported by others (Ma et al., 2014). As a result, the selective pressure will often lead to the development of corresponding resistance determinants that facilitate evasion of the inhibitory substances.

So far, little is known about the status of integrons and related gene cassettes of broiler-originated *Campylobacter* in China as well as the determinant markers responsible for resistance to fluoroquinolone, tetracycline and erythromycin. In the present study, we reported the investigation of a large random collection of MDR *Campylobacter* for the presence of class 1 integrons. Consequently, 98.6% of the campylobacters possessed class 1 integrons, and 52.7% (78/148) of the isolates were positive for the corresponding gene cassettes. According to the results, three distinct amplicon profiles were confirmed and 1000bp-sized amplicons were investigated in an attempt to identify any potential coding sequences. Seventy eight isolates were associated with this particular amplicon which were present in both *C. jejuni* (16.7%) and *C. coli* (83.3%) isolates. Interestingly, this group predominantly consisted of *C. coli* species (83.3%, 65/78) isolated from cecal samples and carcasses, which attach an interpretation for the higher prevalence of MDR *C. coli*. Characterization of the 1000bp amplicon in randomly selected isolates ($n = 20$) independently accounted for highly similar *aadA2*-encoding gene cassettes from *C. jejuni* and *C. coli*. This finding demonstrates that identical class 1 integron structures are present in different members of the same genus, suggesting that genetic exchange may have occurred in the gastrointestinal environment and plays an important role in the horizontal dissemination of antimicrobial resistance among the bacteria. Furthermore, the presence of class 1 integrons in several Campylobacter isolates may partly contribute to the high levels of resistance to streptomycin and gentamycin.

The presence of *tetO* gene was detected in 94.6% (123/130) of tetracycline-resistant *Campylobacter* isolates. The high occurrence of *tetO* genes in these isolates represents a potential high tetracycline resistance in *Campylobacter* isolates obtained from poultry in China, because the *tetO* gene is the most commonly reported determinant conferring resistance to tetracycline in the *Campylobacter* genus, and in many previous study, this gene was detected in



Strains	Genotypes	AMR patterns	Resistance determinants				
			A	B	C	D	E
13-JF89	1 JF-CC	CIP LEV GEN TET CLI EM	+	-	+	+	+
12-JF4	2 JF-CC	CIP LEV TET	+	aadA2	+	+	+
R11	3 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	-	+	+
1-1	3 JH-CC	CIP LEV GEN TET CLI EM FLO	+	aadA2	+	+	+
1-28	3 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF8	3 JF-CC	CIP LEV TET CLI FLO	+	aadA2	+	+	+
R9	3 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF14	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF23	4 JF-CC	CIP LEV TET CLI	+	aadA2	+	+	+
13-JF13	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF12	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-21	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	-	+	+
1-18	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF2	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R16	4 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R5	4 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-31	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	-	+
1-30	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	-	+	+
1-15	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-12	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	-	+	+
R15	4 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R17	4 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-29	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-25	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-3	4 JH-CC	CIP LEV TET CLI EM FLO	+	aadA2	+	+	+
1-27	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	-	+
13-JF6	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-24	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF5	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF1	4 JF-CC	CIP LEV GEN STR TET CLI EM	+	aadA2	+	+	+
R14	4 JR-CC	CIP LEV GEN TET CLI EM FLO	+	aadA2	+	+	+
13-JF7	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF16	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	-	+	+
13-JF10	4 JF-CC	CIP LEV TET CLI FLO	+	aadA2	+	+	+
13-JF11	4 JF-CC	CIP LEV TET CLI FLO	+	aadA2	+	+	+
13-JF17	4 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-26	4 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF15	5 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	-
1-23	6 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-13	6 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-19	7 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF4	8 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-16	8 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R7	8 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
13-JF3	8 JF-CC	CIP LEV GEN STR TET CLI EM	+	aadA2	+	+	+
1-6	9 JH-CC	CIP LEV TET CLI	+	aadA2	+	+	+
1-2	9 JH-CC	CIP LEV GEN STR TET CLI EM FLO	+	aadA2	+	+	+
1-7	9 JH-CC	CIP LEV TET CLI EM FLO	+	aadA2	+	+	+
12-JF3	9 JF-CC	CIP LEV GEN STR TET CLI EM	+	-	+	-	+
1-17	9 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
12-JF91	10 JF-CC	CIP LEV GEN STR TET CLI EM	-	-	-	-	-
R1	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R2	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R13	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R12	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R6	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R8	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R10	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
R3	11 JR-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
12-JF13	11 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-20	11 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	-	+	+
1-22	11 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-10	11 JH-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+
1-4	12 JH-CC	CIP LEV GEN STR TET CLI EM	+	aadA2	+	+	+
R4	12 JR-CC	CIP LEV GEN TET CLI EM FLO	+	aadA2	+	+	+
13-JF9	13 JF-CC	CIP LEV GEN STR TET CLI EM	+	aadA2	+	+	+
13-JF47	14 JF-CC	CIP LEV TET CLI EM	+	aadA2	+	+	+
13-JF56	15 JF-CC	CIP LEV GEN TET CLI EM	+	aadA2	+	+	+

Fig. 2. Dendrogram of *Sma*I PFGE types, antimicrobial resistance (AMR) pattern and determinant mutations of *C. coli* isolates recovered from cecal samples, carcasses and carcass parts at the slaughterhouse. 1–15, PFGE patterns. JF, cecal samples; JH, carcasses; JR, carcass parts. A, integron 1; B, gene cassettes; C, *gyrA*; D, *tetO*; E, 23S rRNA. +, positive; −, negative. CIP, ciprofloxacin; STR, streptomycin; GEN, gentamicin; FLO, florfenicol; TET, tetracycline; CLI, clindamycin; EM, erythromycin; LEV, levofloxacin.

all tetracycline-resistant *Campylobacter* spp. isolates (Perez-Boto, Herrera-Leon, Garcia-Pena, Abad-Moreno, & Echeita, 2014; Qin et al., 2011).

Macrolide resistance in *Campylobacter* spp. has been mostly linked with *C. coli* isolates of swine origin (Qin et al., 2011). Poultry isolates seem to be more susceptible to this family of antimicrobials, because macrolides are not so commonly used in antibiotic treatment of broilers as in swine. In this study, only 14.3% of *C. jejuni* isolates were resistant to erythromycin, however a relatively high resistance was found among *C. coli* isolates, with a high resistance level of 93.9%. Similar results were reported that *C. coli* showed a significantly higher resistance to erythromycin than *C. jejuni* (Duarte et al., 2014; Ma et al., 2014; Torralbo et al., 2015). This phenomenon may suggest that *C. coli* was more robust than *C. jejuni*

under the selective pressure or imply a potential transmission of drug resistance spreading across different hosts among *C. coli* isolates in the natural environments.

The MAMA-PCR, providing a simple and rapid tool to screen for *Campylobacter* isolates resistant to fluoroquinolones, is proved to have 100% agreement with MIC results and DNA sequence analysis (Alonso et al., 2005; Zirnstein et al., 1999). As a result of this study, 93.1% of ciprofloxacin-resistant *Campylobacter* had a substitution at amino acid position 86 of the *gyrA* protein due to a point mutation of the DNA codon from ACA (threonine) to ATA (isoleucine). Totally, 97.3% (71/73) of *C. jejuni* isolates and 89.7% of *C. coli* isolates (61/68) possessed a Thr-86-Ile substitution in *gyrA*, indicating that this mutation is more prevalent in *C. jejuni* than in *C. coli*. However, other fluoroquinolone resistant strains showed absence of this kind of mutation, which might be explained by other mechanisms including decreased outer membrane permeability (Köhler & Pechère, 2000) and an efflux system (Hungaro et al., 2015; Corcoran, Quinn, Cotter, & Fanning, 2005).

As one of the most discriminatory molecular epidemiological tools available for bacterial fingerprinting, PFGE profiles could indicate the occurrence of cross-contamination events at the slaughterhouse, where could enable the survival of different *Campylobacter* strains. In the present study, we found *Campylobacter* isolates on carcasses and/or carcass parts with identical macrorestriction profiles like those detected in cecal samples. The presence of several shared pulsotypes between cecal samples and carcasses surfaces indicates that fecal contamination at slaughter was frequent. For instance, the 4-CC and 11-CC pulsotype was observed in all samples (cecal contents, carcasses and carcass parts) originating from broilers reared in the same farm and slaughtered on the same day. It has been previously reported that campylobacter-negative flocks were contaminated with campylobacters during transport and slaughter (Peyrat, Soumet, Maris, & Sanders, 2008; Takahashi, Shahada, Chuma, & Okamoto, 2006). For another, it could be possible that *Campylobacter* isolates on carcasses and parts with identical genotypes like those detected in cecal samples were farm-specific isolates as shown for housed broiler flocks in a previous study (Klein, Beckmann, Vollmer, & Bartelt, 2007). Whereas, the isolates of *C. jejuni* which were mainly recovered from cecal samples and showed higher genetic diversity among strains were not isolated in the cut-up stage, and this may suggest that *C. jejuni* is much easier to enter the viable but non-culturable (VBNC) state than *C. coli* under the stress conditions.

Campylobacter isolates with genotype patterns (6-CC, 7-CC) other than those found in cecal samples could be an indication of several contamination sources, e.g. strains from previously processed flocks, if they persist in the wet and hot atmosphere of the slaughterhouse, contaminated carcasses of the examined chickens. In addition, other authors have suggested that campylobacters could be present in aerosols in different areas of the slaughterhouse, including hanging, defeathering, evisceration and chilling (Melero et al., 2012). Furthermore, some strains are able to form biofilms in vitro (Gunther Iv & Chen, 2009), and some can survive longer and resist inactivation by disinfectants in pre-existing biofilms formed by other microorganisms (Trachoo & Frank, 2002).

In conclusion, a relatively high level of contamination with *Campylobacter* spp. was present in the slaughterhouse, thus the maintenance of processing hygiene and regular microbiological inspection of carcasses are essential to minimize the cross-contamination. Besides, the antimicrobial resistance is common among *Campylobacter* strains from broilers in China, which calls for rational use of drugs and continued monitoring during the rearing stage. It is also important to raise consumer's awareness of correct handling and cooking of poultry meats to avoid cross contamination at home before consumption.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2016.04.051.

References

Abdollahpour, N., Zendehbad, B., Alipour, A., & Khayatzadeh, J. (2015). Wild-bird feces as a source of *Campylobacter jejuni* infection in children's playgrounds in Iran. *Food Control, 50*(0), 378–381.

Allos, B. M. (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. *Clinical Infectious Diseases, 32*(8), 1201–1206.

Alonso, R., Mateo, E., Churruca, E., Martinez, I., Girbau, C., & Fernandez-Astorga, A. (2005). MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli* strains. *Journal of Microbiological Methods, 63*(1), 99–103.

Andrzejewska, M., Szczepańska, B., Śpica, D., & Klawe, J. J. (2015). Trends in the occurrence and characteristics of *Campylobacter jejuni* and *Campylobacter coli* isolates from poultry meat in Northern Poland. *Food Control, 51*(0), 190–194.

Bakeli, G., Sato, K., Kumita, W., Saito, R., Ono, E., Chida, T., et al. (2008). Antimicrobial susceptibility and mechanism of quinolone resistance in *Campylobacter jejuni* strains isolated from diarrheal patients in a hospital in Tokyo. *Journal of Infection and Chemotherapy, 14*(5), 342–348.

Chen, X., Naren, G.-W., Wu, C.-M., Wang, Y., Dai, L., Xia, L.-N., et al. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Veterinary Microbiology, 144*(1–2), 133–139.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*(2), 232–260.

Clinical and Laboratory Standards Institute. (2010). *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved guideline-Second edition. M45–A2*. Wayne, PA: Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standards Institute. (2012). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard-Ninth edition* (pp. M07–A9). Wayne, PA: Clinical and Laboratory Standards Institute.

Corcoran, D., Quinn, T., Cotter, L., & Fanning, S. (2005). Relative contribution of target gene mutation and efflux to varying quinolone resistance in Irish *Campylobacter* isolates. *FEMS Microbiology Letters, 253*(1), 39–46.

Dallal, M. M. S., Doyle, M. P., Rezadehbashi, M., Dabiri, H., Sanaei, M., Modarresi, S., et al. (2010). Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, *Campylobacter* and *Yersinia* spp. isolated from retail chicken and beef, Tehran, Iran. *Food Control, 21*(4), 388–392.

Denis, M., Chidaine, B., Laisney, M. J., Kempf, I., Rivoal, K., Megraud, F., et al. (2009). Comparison of genetic profiles of *Campylobacter* strains isolated from poultry, pig and *Campylobacter* human infections in Brittany, France. *Pathologie Biologie (Paris), 57*(1), 23–29.

Denis, M., Soumet, C., Rivoal, K., Ermel, G., & Blivet, D. (1999). Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Letters in Applied Microbiology, 29*(6), 406–410.

Duarte, A., Santos, A., Manageiro, V., Martins, A., Fraqueza, M. J., Caniça, M., et al. (2014). Human, food and animal *Campylobacter* spp. isolated in Portugal: high genetic diversity and antibiotic resistance rates. *International Journal of Antimicrobial Agents, 44*(4), 306–313.

Economou, V., Zisides, N., Gousia, P., Petsios, S., Sakkas, H., Soultos, N., et al. (2015). Prevalence and antimicrobial profile of *Campylobacter* isolates from free-range and conventional farming chicken meat during a 6-year survey. *Food Control, 56*(0), 161–168.

Ekkapobyotin, C., Padungtod, P., & Chuanchuen, R. (2008). Antimicrobial resistance of *Campylobacter coli* isolates from swine. *International Journal of Food Microbiology, 128*(2), 325–328.

Ellerbroek, L. I., Lienau, J. A., & Klein, G. (2010). *Campylobacter* spp. in broiler flocks at farm level and the potential for cross-contamination during slaughter. *Zoonoses Public Health, 57*(7–8), e81–88.

van Essen-Zandbergen, A., Smith, H., Veldman, K., & Mevius, D. (2007). Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli, Salmonella* and *Campylobacter* spp. in the Netherlands. *The Journal of Antimicrobial Chemotherapy, 59*(4), 746–750.

van Gerwe, T., Bouma, A., Wagenaar, J. A., Jacobs-Reitsma, W. F., & Stegeman, A. (2010). Comparison of *Campylobacter* levels in crops and ceca of broilers at slaughter. *Avian Diseases, 54*(3), 1072–1074.

Ge, B., Wang, F., Sjolund-Karlsson, M., & McDermott, P. F. (2013). Antimicrobial

resistance in campylobacter: susceptibility testing methods and resistance trends. *Journal of Microbiological Methods, 95*(1), 57–67.
Gibreel, A., Tracz, D. M., Nonaka, L., Ngo, T. M., Connell, S. R., & Taylor, D. E. (2004). Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance. *Antimicrobial Agents and Chemotherapy, 48*(9), 3442–3450.
Guyard-Nicodème, M., Rivoal, K., Houard, E., Rose, V., Quesne, S., Mourand, G., et al. (2015). Prevalence and characterization of *Campylobacter jejuni* from chicken meat sold in French retail outlets. *International Journal of Food Microbiology, 203*(0), 8–14.
Hariharan, H., Sharma, S., Chikweto, A., Matthew, V., & DeAllie, C. (2009). Antimicrobial drug resistance as determined by the E-test in *Campylobacter jejuni, C. coli*, and *C. lari* isolates from the ceca of broiler and layer chickens in Grenada. *Comparative Immunology Microbiology and Infectious Diseases, 32*(1), 21–28.
Henry, I., Reichardt, J., Denis, M., & Cardinale, E. (2011). Prevalence and risk factors for *Campylobacter* spp. in chicken broiler flocks in Reunion Island (Indian Ocean). *Preventive Veterinary Medicine, 100*(1), 64–70.
Humphrey, T., O'Brien, S., & Madsen, M. (2007). *Campylobacters* as zoonotic pathogens: a food production perspective. *International Journal of Food Microbiology, 117*(3), 237–257.
Hungaro, H. M., Mendonça, R. C. S., Rosa, V. O., Badaró, A. C. L., Moreira, M. A. S., & Chaves, J. B. P. (2015). Low contamination of *Campylobacter* spp. on chicken carcasses in Minas Gerais state, Brazil: molecular characterization and antimicrobial resistance. *Food Control, 51*(0), 15–22.
Iovine, N. M. (2013). Resistance mechanisms in *Campylobacter jejuni*. *Virulence, 4*(3), 230–240.
Gunther Iv, N. W., & Chen, C.-Y. (2009). The biofilm forming potential of bacterial species in the genus *Campylobacter*. *Food Microbiology, 26*(1), 44–51.
Kassa, T., Gebre-Selassie, S., & Asrat, D. (2007). Antimicrobial susceptibility patterns of thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia. *Veterinary Microbiology, 119*(1), 82–87.
Klein, G., Beckmann, L., Vollmer, H. M., & Bartelt, E. (2007). Predominant strains of thermophilic *Campylobacter* spp. in a german poultry slaughterhouse. *International Journal of Food Microbiology, 117*(3), 324–328.
Köhler, T., & Pechère, J.-C. (2000). Chapter 4-bacterial resistance to quinolones: mechanisms and clinical implications. In V. T. Andriole (Ed.), *The quinolones* (3rd ed., pp. 139–167). San Diego: Academic Press.
Kurinčič, M., Botteldoorn, N., Herman, L., & Smole Možina, S. (2007). Mechanisms of erythromycin resistance of *Campylobacter* spp. isolated from food, animals and humans. *International Journal of Food Microbiology, 120*(1–2), 186–190.
Lee, M. D., Sanchez, S., Zimmer, M., Idris, U., Berrang, M. E., & McDermott, P. F. (2002). Class 1 integron-associated Tobramycin-gentamicin resistance in *Campylobacter jejuni* isolated from the broiler chicken house environment. *Antimicrobial Agents and Chemotherapy, 46*(11), 3660–3664.
Lévesque, C., Piché, L., Larose, C., & Roy, P. H. (2005). PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrobial Agents and Chemotherapy, 39*(1), 185–191.
Ma, L., Wang, Y., Shen, J., Zhang, Q., & Wu, C. (2014). Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China. *International Journal of Food Microbiology, 181*, 77–84.
Melero, B., Juntunen, P., Hänninen, M.-L., Jaime, I., & Rovira, J. (2012). Tracing *Campylobacter jejuni* strains along the poultry meat production chain from farm to retail by pulsed-field gel electrophoresis, and the antimicrobial resistance of isolates. *Food Microbiology, 32*(1), 124–128.
O'Halloran, F., Lucey, B., Cryan, B., Buckley, T., & Fanning, S. (2004). Molecular characterization of class 1 integrons from Irish thermophilic *Campylobacter* spp. *The Journal of Antimicrobial Chemotherapy, 53*(6), 952–957.
Payot, S., Dridi, S., Laroche, M., Federighi, M., & Magras, C. (2004). Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in France. *Veterinary Microbiology, 101*(2), 91–99.
Pepe, T., De Dominicis, R., Esposito, G., Ventrone, I., Fratamico, P. M., & Cortesi, M. L. (2009). Detection of *Campylobacter* from poultry carcass skin samples at slaughter in Southern Italy. *Journal of Food Protection, 72*(8), 1718–1721.
Perez-Boto, D., Herrera-Leon, S., Garcia-Pena, F. J., Abad-Moreno, J. C., & Echeita, M. A. (2014). Molecular mechanisms of quinolone, macrolide, and tetracycline resistance among *Campylobacter* isolates from initial stages of broiler production. *Avian Pathology, 43*(2), 176–182.
Perez-Perez, G. I., & Kienesberger, S. (2013). Chapter 9-*Campylobacter*. In J. G. M. E. Potter (Ed.), *Foodborne infections and intoxications* (4th ed., pp. 165–185). San Diego: Academic Press.
Peyrat, M. B., Soumet, C., Maris, P., & Sanders, P. (2008). Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: analysis of a potential source of carcass contamination. *International Journal of Food Microbiology, 124*(2), 188–194.
Piccirillo, A., Dotto, G., Salata, C., & Giacomelli, M. (2013). Absence of class 1 and class 2 integrons among *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in Italy. *The Journal of Antimicrobial Chemotherapy, 68*(11), 2683–2685.
Qin, S.-S., Wu, C.-M., Wang, Y., Jeon, B., Shen, Z.-Q., Wang, Y., et al. (2011). Antimicrobial resistance in *Campylobacter coli* isolated from pigs in two provinces of China. *International Journal of Food Microbiology, 146*(1), 94–98.
Rasschaert, G., Houf, K., Van Hende, J., & De Zutter, L. (2006). *Campylobacter* contamination during poultry slaughter in Belgium. *Journal of Food Protection, 69*(1), 27–33.
Rowe, M. T., & Madden, R. H. (2014). CAMPYLOBACTER | introduction. In C. A. B. L. Tortorello (Ed.), *Encyclopedia of food microbiology* (2nd ed., pp. 351–356). Oxford: Academic Press.
Ruiz-Castillo, A., Torres-Sánchez, M. J., & Aznar-Martín, J. (2014). Molecular epidemiology and antimicrobial susceptibility of *Campylobacter coli* clinical isolates. *Enfermedades Infecciosas y Microbiología Clínica, 32*(7), 443–445.
Sahin, O., Plummer, P. J., Jordan, D. M., Sulaj, K., Pereira, S., Robbe-Austerman, S., et al. (2008). Emergence of a tetracycline-resistant *Campylobacter jejuni* clone associated with outbreaks of ovine abortion in the United States. *Journal of Clinical Microbiology, 46*(5), 1663–1671.
Sproston, E. L., Carrillo, C. D., & Boulter-Bitzer, J. (2014). The quantitative and qualitative recovery of *Campylobacter* from raw poultry using USDA and Health Canada methods. *Food Microbiology, 44*(0), 258–263.
Szczepanska, B., Kaminski, P., Andrzejewska, M., Spica, D., Kartanas, E., Ulrich, W., et al. (2015). Prevalence, virulence, and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* in white stork Ciconia ciconia in Poland. *Foodborne Pathog Dis, 12*(1), 24–31.
Taboada, E. N., Clark, C. G., Sproston, E. L., & Carrillo, C. D. (2013). Current methods for molecular typing of *Campylobacter* species. *Journal of Microbiological Methods, 95*(1), 24–31.
Takahashi, R., Shahada, F., Chuma, T., & Okamoto, K. (2006). Analysis of *Campylobacter* spp. contamination in broilers from the farm to the final meat cuts by using restriction fragment length polymorphism of the polymerase chain reaction products. *International Journal of Food Microbiology, 110*(3), 240–245.
Torralbo, A., Borge, C., Garcia-Bocanegra, I., Meric, G., Perea, A., & Carbonero, A. (2015). Higher resistance of *Campylobacter coli* compared to *Campylobacter jejuni* at chicken slaughterhouse. *Comparative Immunology Microbiology and Infectious Diseases, 39*, 47–52.
Trachoo, N., & Frank, J. F. (2002). Effectiveness of chemical sanitizers against *Campylobacter jejuni*-containing biofilms. *Journal of Food Protection, 65*(7), 1117–1121.
Wieczorek, K., Denis, E., Lynch, O., & Osek, J. (2013). Molecular characterization and antibiotic resistance profiling of *Campylobacter* isolated from cattle in Polish slaughterhouses. *Food Microbiology, 34*(1), 130–136.
Zendehbad, B., Khayatzadeh, J., & Alipour, A. (2015). Prevalence, seasonality and antibiotic susceptibility of *Campylobacter* spp. isolates of retail broiler meat in Iran. *Food Control, 53*(0), 41–45.
Zhang, M., Liu, X., Xu, X., Gu, Y., Tao, X., Yang, X., et al. (2014). Molecular subtyping and antimicrobial susceptibilities of *Campylobacter coli* isolates from diarrheal patients and food-producing animals in China. *Foodborne Pathogens and Diseases, 11*(8), 610–619.
Zirnstein, G., YU, L., Swaminathan, B., & Angulo, F. (1999). Ciprofloxacin resistance in *Campylobacter jejuni* isolates detection of *gyrA* resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. *Journal of Clinical Microbiology, 37*(10), 3276–3280.