ABSTRACT

QIU, YUCAN. Antimicrobial Susceptibility and Clonal Population Structure of Multidrug Resistant *Campylobacter jejuni* Isolates from Commercial Turkeys in North Carolina. (Under the direction of Dr. Sophia Kathariou).

Campylobacter is one of the leading causes of human bacterial gastroenteritis in the industrialized world and contaminated poultry are a leading vehicle for disease. Commercial turkey flocks in eastern North Carolina have been found to be frequently colonized with *C. jejuni* that are resistant to several antimicrobials (tetracycline, streptomycin, kanamycin, and nalidixic acid/ciprofloxacin) and have been designated multidrug resistant (MDR). In this study, we assessed the prevalence of MDR *C. jejuni* in North Carolina between 2008 and 2012. Turkeys were from 467 flocks, operated by four different companies (integrators). Prevalence assessments suggest high prevalence of MDR *C. jejuni* in both brooders and grow-out turkeys. A total of 141 *C. jejuni* isolates were characterized by pulsed-field gel electrophoresis (PFGE) with SmaI. The majority (67%) of these isolates had the MDR profile, and the remainder (33%) was susceptible to just one or two of the antibiotics mentioned above. PFGE analysis revealed that MDR *C. jejuni* were genetically distinct from non-MDR *C. jejuni*. Bionumerics-based cluster analysis of PFGE profiles of MDR *C. jejuni* yielded three main clusters of isolates, of which one (cluster A2) included most of the MDR isolates. Multilocus sequence typing (MLST) of 5 MDR *C. jejuni* isolates representing all three clusters identified 2 sequence types (ST1839 and the closely related ST2936). Both STs were turkey-specific. The ST1839 was predominant (four of

the five isolates) and was detected among isolates from different farms. Analysis of MIC determinations revealed that MDR *C. jejuni* were highly resistant to roxarsone, and were inhibited at relatively low concentrations of gentamicin and benzalkonium chloride. The findings from this study suggest high prevalence of certain closely related clonal groups of MDR *C. jejuni* in turkey production in this region. Further studies are required to identify and characterize the possible routes of transmission of these clonal groups of MDR *C. jejuni* in the turkey industry.

Antimicrobial Susceptibility and Clonal Population Structure of Multidrug Resistant *Campylobacter jejuni* Isolates from Commercial Turkeys in North Carolina

by
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DEDICATION

I dedicate this work to my loving parents.

BIOGRAPHY

Yucan Qiu was born on March 19, 1989 in Shaoxing, Zhejiang Province, P. R. China. Yucan graduated from Hangzhou High School. He continued his education in the College of Agricultural and Biotechnology in Zhejiang University, which is one of the best universities in China. He majored in Tea Science and completed his thesis “Comparison in absorption of fluorine between different types of tea plant & Diversity of tea plants of low fluoride absorption” under direction of Dr. Yuerong Liang. In addition, Yucan won the General Department Scholarship, Scholarship for Excellent Academic Work in his undergraduate study and earned a Bachelor of Science in June 2011. After that, He pursued his interest and continued his education as a Non-thesis Master student majored in Food Science in North Carolina State University, Raleigh, NC, USA through the "3+X" program. After one year of study, Yucan changed to M.S. degree under direction of Dr. Sophia Kathariou.

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CHAPTER I:

Literature Review

1.1 Introduction

Campylobacter has been recognized as a leading cause of human acute bacterial foodborne disease in many countries since the late 1970s (Blaser et al., 1983). *Campylobacter* cells are small, motile, gram negative rods with a polar flagellum at one or both ends of the cell (Ketley et al., 2001). It has a characteristic curved or spiral morphology with size from 0.2 to 0.8 μm wide and 0.5 to 5μm long in its normal state, but the cells become coccoid in older cultures especially on solid media, and this form has been suggested to be a dormant state required for survival under conditions not supporting growth of *Campylobacter* (Buck et al., 1983; Debruyne et al., 2008). *Campylobacter* is a fastidious bacterium in the laboratory. *C. jejuni* and *C. coli* are considered to be the thermophilic *Campylobacter* species whose temperature range for growth is between 34 to 44℃, with an optimal growth temperature at 42 ℃ (Ketley et al., 2001). *Campylobacter* is an obligate microaerophile, which requires oxygen concentration at 3 to 5%, and carbon dioxide concentration at 3 to 5% (Ketley et al., 2001). *Campylobacter* is capable of surviving in the pH range of 4.0 to 9.0, and grows optimally at pH 6.5 to 7.5. On freshly made media, *Campylobacter* colonies are normally grey, flat, irregular and spreading (Nachamkin et al., 2000).

Hippurate hydrolysis is a test used to differentiate between *C. jejuni* and *C. coli*, which detects glycine that is an end-product of the hydrolysis of hippurate. The positive hippurate hydrolysis result indicates that the *Campylobacter* is *C. jejuni* (Skirrow and

Benjamin, 1980). PCR methods employing specific primers targeting the *hipO* gene is used to positively identify *C. jejuni*, and for *C. coli* identification, PCR primers based on the *ceuE* gene are used (Hani and Chan, 1995; Gonzalez et al., 1997).

The vast majority of *Campylobacter* infections are isolated, sporadic events, not as part of recognized outbreaks (Friedman et al., 2000). Poultry, raw milk, water, pets, and wild birds have been suggested as sources of *Campylobacter* infections (Fujimoto et al., 1997). In industrialized countries, the incidence of *Campylobacter* infections is as high as 1% of the population per year. In the United States alone, in spite of many undiagnosed or unreported cases, there is still an estimated 0.8 million *Campylobacter* infections every year, associated with an estimated 124 deaths (CDC, 2010). Recent US Centers for Disease Control and Prevention (CDC) Foodborne Disease Surveillance Network (FoodNet) data suggest that *Campylobacter* accounts for about 13 infections per 100,000 persons (Scallan et al., 2011). *Campylobacter* infection is usually a self-limiting diarrheal disease, but can also have severe long-term sequelae such as reactive arthritis, and Guillain–Barré syndrome (Humphrey et al., 2007). The symptoms of *Campylobacter* infection are more severe in developed countries than in developing countries (Oberhelman and Taylor, 2000). *Campylobacter* infections are especially high among children less than 5 years of age (Scallan et al., 2011). In industrialized countries, it is estimated that the majority (80% to 90%) of the *Campylobacter* infections cases involve *C. jejuni*, with most of the remainder being due to *C. coli* (Friedman et al., 2000; Nachamkin et al., 2000).

1.2 Genome of *Campylobacter*

The genome size of *Campylobacter* is approximately 1.7 megabases (Mb) (Derrick et al., 2005). Among the *Campylobacter* species, the major differences in the genomes involve inserted phage or genomic islands (Derrick et al., 2005). Genome sequencing has revealed that there are around twenty regions that are hypervariable among different *C. jejuni* strains (Derrick et al., 2005). These diversified regions include four large genomic elements called *Campylobacter jejuni*-integrated elements (CJIEs) and more than 18 loci inivolved in lipooligosaccharide (LOS) biosynthesis, capsular polysaccharide (CPS) biosynthesis, flagellar modification and DNA restriction/modification systems (Parker et al., 2006).

The genomes of several *C. jejuni* strains including NCTC1168 (genome size 1.6Mbp), RM 1221 (genome size 1.8Mbps), and NCTC11828 (genome size 1.6Mbps) have been completely sequenced (Derrick et al., 2005; Parkhill et al., 2000). The genome of *C. coli* strain RM 2228 was also sequenced, but sequence gaps have not been fully closed (Derrick et al., 2005). The genomes of the *C. jejuni* strains appear to more closely relate to each other than to *C. coli*. *C. jejuni* RM 1221 has an average 98.41% protein percent identity with *C.jejuni* NCTC11168, while an average of 85.81% protein identity with C. coli RM2228 (Derrick et al., 2005).

Campylobacter is able to take up DNA from the environment (i.e. is competent for transformation), resulting in recombination between genomes from different strains. The

genetic exchange mechanisms of *Campylobacter* include natural transformation and conjugation. The capacity of horizontal transfer of DNA plays an important role in introduction of new factors like antibiotic resistance in *Campylobacter* through transformation (Wilson et al., 2003).

1.3 Genotyping of *Campylobacter*

1.3.1 Flagellin typing (*fla* typing): *Campylobacter* is motile microorganism mainly due to it has a polar flagellum at one or both ends of the cell. The flagellin filament is encoded by two flagellin genes, *flaA* and *flaB*, each about 1.7kb and separated from each other by approximately 170bp (Guerry et al., 1991). The flagellin genes in *Campylobacter* are heterogeneous enough to be used in genotyping schemes such as PCR- based Restriction Fragment Length Polymorphism (RFLP) (Derrick et al., 2005; Meinersmann et al., 1997). The primers were designed to bind the regions which are conserved in *C. jejuni* and are partially conserved in other *Campylobacter* species (Meinersmann et al., 1997). The result of *fla* typing can be visualized by using gel electrophoresis. *Fla* typing has proved to be a useful technique for epidemiological studies and strain discrimination in an outbreak analysis (Wassenaar and Newell, 2000, On et al., 2008).

1.3.2 Pulsed-Field Gel Electrophoresis (PFGE): PFGE is one of the most commonly used discriminatory typing methods for genotyping or genetic fingerprinting of bacteria (On et al., 2008). PFGE is based on the presence of restriction recognition sites throughout the genome and involves the use of restriction enzymes to produce large fragments. Different

restriction enzymes were chosen to cut infrequently so that large fragments could be formed and then separated by gel electrophoresis in an electric filed that is changed in a pulsing manner (Wassenaar and Newell, 2000). Strains belonging to the same *fla* typing can be differentiated by PFGE (Leatherbarrow et al., 2004).

1.3.3 Multilocus sequence typing (MLST): MLST is a DNA sequence-based genotyping method. This method is based on the sequence of internal fragments of the seven housekeeping genes: *aspA*, *glnA*, *gltA*, *glyA*, *atpA*, *pgm*, and *tkt* (Dingle et al., 2001). For each gene, different sequences are assigned as distinct alleles (Dingle et al., 2001). Miller et al. has used this technique to identify host-associated alleles in *C. coli* isolates from food animals, and found that the majority of sequence types and alleles were host-associated (Miller et al., 2006).

1.4. Antibiotic resistance

Bacteria develop antibiotic resistance either by mutation or by achieving the specific resistance gens from exogenous sources (Davies, 1994). Although the majority of human *Campylobacter* infections are self-limiting, macrolides and fluoroquinolones are still commonly used to treat infections (Engberg et al., 2001). Antibiotics such as tetracycline and tylosin are widely used in food animals for disease treatment or as growth promoters (Lee et al., 2005).

Numerous studies have been done to investigate the emergence of antimicrobial resistance in *C. jejuni* and *C. coli* isolated from both humans and food animals in many

countries around the world. In New Zealand, a study of 202 enteric isolates from Auckland reported that antibiotic resistance was generally uncommon in 1998, with no resistance detected to gentamicin, and with 1%, 1.5% and 2.5% resistance to doxycycline, erythromycin and ciprofloxacin, respectively (Dowling et al., 1998). However, three years later, a study of 200 isolates from the same area of New Zealand found that erythromycin resistance and ciprofloxacin resistance were more common in *C. jejuni*, with 3% of *C. jejuni* isolates resistant to erythromycin and 4% to ciprofloxacin (Goodchild et al., 2001). In Australia, a study that included 88 chicken strains reported that 31% of *C. jejuni* and 22% of *C. coli* were resistant to erythromycin, 10% of *C. jejuni* and 33% of *C. coli* were resistant to doxycycline, and no isolates were detected resistant to enrofloxacin (Korolik et al, 1996). In France, 15 to 35% of *C. jejuni* isolated from broilers were resistant to ampicillin, 25 to 44% were resistant to nalidixic acid, 17 to 32% were resistant to fluoroquinolones, 55 to 68% were resistant to tetracycline, 0 to 5% were resistant to erythromycin, and no isolates were resistant to gentamicin (Moore et al., 2006). Moreover, for *C. coli* isolated from broilers, 25 to 31% were resistant to ampicillin, 40 to 44% were resistant to nalidixic acid, 29 to 41% were resistant to fluoroquinolones, 60 to 97% were resistant to tetracycline, 11 to 31% were resistant to erythromycin, and no isolates were resistant to gentamicin; for *C. coli* isolated from swine, 11 to 15% were resistant to ampicillin, 19 to 38% were resistant to nalidixic acid, 11 to 24% were resistant to fluoroquinolones, 83 to 96% were resistant to tetracycline, 48 to 78% were resistant to erythromycin, and no isolates were resistant to

gentamicin (Moore et al., 2006). These data suggested that ampicillin, quinolone, fluoroquinolones and tetracycline may not be as effective as erythromycin and gentamicin in controlling *C. jejuni* and *C. coli*.

The majority of studies on poultry have focused on broilers, with relatively limited information on turkeys. Luangtongkum et al. reported that although *Campylobacter* species were highly prevalent in both conventional and organic poultry operations, the antimicrobial resistance rates were significantly different between organic and conventional operations (Luangtongkum et al., 2006). The conventionally raised poultry demonstrated much higher prevalence of antimicrobial resistance than organically raised poultry, and the differences were obvious with fluoroquinolones (Luangtongkum et al., 2006). An estimated 46% and 67% of isolates from conventionally raised broilers and conventionally raised turkeys were resistant to fluoroquinolones, while less than 2% of isolates from organically raised poultry were resistant (Luangtongkum et al., 2006). Moreover, multidrug resistance was also mainly observed among *Campylobacter* strains isolated from the conventional turkey operation (Luangtongkum et al., 2006).

Harun Ahmad's study showed that most multidrug resistant *C. coli* in North Carolina were not inhibited at the highest used concentration of ampicilin (92.8%), erythromycin (99.3%), kanamycin (98.7%), and streptomycin (99.3%) (Harun, 2011). A correlation between levels of resistance to fluoroquinolones and to tetracycline was found in this study (Harun, 2011).

Gu et al. reported that *C. jejuni* in eastern North Carolina had high prevalence (60% to ca. 98%) of resistance to ampicillin, tetracycline, streptomycin, and nalidixic acid/ciprofloxacin (Gu et al., 2009). The majority of the *C. jejuni* isolates (74%) were resistant to three or more antimicrobials, but the resistance to erythromycin was never encountered (Gu et al., 2009). It also revealed a strong correlation between kanamycin resistance and tetracycline resistance: most kanamycin-resistant isolates were also resistant to tetracycline; however, the reverse was not true (Gu et al., 2009).

1.5 Transmission of *Campylobacter*

Numerous studies have investigated the potential for vertical transmission; carry over from previous flocks, and horizontal transmission via contaminated water, domestic and wild animals, personnel working in the broiler house, and the external environment (Zweifel et al., 2008; Humphrey et al., 1993; Berndston et al., 1996). In the study of Cox et al, one broiler breeder flock in Georgia and one in Arkansas were surveyed for prevalence of *Campylobacter* colonization; results demonstrated that *Campylobacter* isolates from breeder flocks and those from the respective broiler progeny had identical genetic patterns, which indicated the occurrence of vertical transmission, i.e that the breeder hens can potentially serve as a source for *Campylobacter* contamination of poultry flocks (Cox et al., 2002). However, most previous studies suggested that vertical transmission of *Campylobacter* from previous colonized breeder flocks is not a likely route of infection (Jacobs-Reitsma, 1997; Humphrey et al., 1993; Shanker et al., 1986). In a British study in

23 farms, all hatchery waste samples were *Campylobacter*- negative and there was a delay of up to 3 weeks before chicks became *Campylobacter*-positive (Humphrey et al., 1993). In the study of Pearson et al, none of the newly hatched chicks or eggs tested was infected by *Campylobacter*, which indicated that there was no vertical transmission of *Campylobacter* (Pearson et al., 1993).

Since viable but non-culturable *Campylobacter* can survive in environmental water, the water supply to farms was suspected as a source of *Campylobacter* for flocks in several studies (Berndston et al., 1996; Pearson et al., 1993). In the study of Pearson et al in Southern England, there was a high colonization rate of chicken with a single serotype of *Campylobacter*, indicating there was an intermittent or continuous common source of *Campylobacter* which was believed to be water (Pearson et al., 1993). A Norwegian study suggested that the contaminated drinking water is an important source of *Campylobacter* for broiler flocks. Using surface water which was not disinfected was strongly associated with an increased risk of colonization, and disinfection of drinking water could effectively reduce the prevalence of *Campylobacter* among broiler chicken flocks (Kapperud et al., 1993). However, water is not always a source of *Campylobacter*. The Humphrey study suggested that there was no evidence that the prevalence of *Campylobacter* colonization was associated with water source (Humphrey et al., 1993). A study of *Campylobacter* in 18 Swedish chicken farms suggested that, under Swedish conditions, water was not a source of *Campylobacter* for chickens since the water distribution system in Sweden gives less

opportunity for *Campylobacter* to survive (Berndston et al., 1996). However, in the Swedish study, it is interesting to see that the water consumption of chickens had an influence on *Campylobacter* infection rate: *Campylobacter* prevalence was 56% in flocks with higher water intake compared with 28% in those with standard water consumption. This may be because the increased water intake could result in wetter litter conditions in flocks which might favor survival of the bacteria (Berndston et al., 1996).

Domestic and wild animals can act as a reservoir of *Campylobacter*. Cattle and other animals on or near poultry farms were found associated with increased risk for *Campylobacter* in several studies (Bouwknegt et al.,2004; Ellis-Iversen et al., 2009; Zweifel et al., 2008; Kapperud et al., 1993). In the Norwegian study mentioned above, the risk of *Campylobacter* colonization in broiler flocks was increased when staff handled other poultry as well as pigs (Kapperud et al., 1993). The Berndston study demonstrated that the management of other domestic animals simultaneously did not increase the risk for *Campylobacter*, but the *Campylobacter* contamination rate increased if staff handled other poultry at the same time; the results show that *Campylobacter* prevalence was 40% on farm with staff handling other poultry, but only 18% if other animals were handled (Berndston et al., 1996). However, some studies suggested that the surrounding domestic animals were not associated with *Campylobacter* prevalence. A study with turkeys grown in close proximity with swine in North Carolina (and commonly by the same farmer) showed that genotypes of turkey and swine-derived *Campylobacter* did not overlap (Wright, 2007).

Several studies also indicated that *Campylobacter* transmission can be via pests, since some pests are able to carry *Campylobacter* and may act as reservoirs on the farm (Berndston et al., 1996; Bemdtson et al., 1994). In the Swedish study, 40% of the flocks were contaminated by *Campylobacter* in flocks where occurrence of mice was noticed compared with 23% of those in flocks where no mice were noticed; 39% of the flocks whose litter samples had darkling beetles were *Campylobacter* positive compared with 26% of those in flocks where no insects were found (Berndston et al., 1996).

Personnel working in the broiler house are a potential route of *Campylobacter* transmission. Several studies demonstrated that the house surroundings can act as a possible source of *Campylobacter* for flocks and two or more people taking care of the house can lead to an increased risk of *Campylobacter* introduction into the house. In a French study, the risk of *Campylobacter* was increased when there were three or more broiler houses on the farm and when two or more persons took care of the flock at the same time (Refre´gier et al., 2001). It is believed that tools or equipment that staff brought into the house or the clothing of the staff can be the source of *Campylobacter*. This would suggest that strict hygiene barriers can reduce *Campylobacter* infection rate effectively. The British study in 23 farms suggested that boot-dipping in strong phenolic disinfectant before entering the house can reduce *Campylobacter* infection: flocks in the houses with boot-dipping measures remained uncolonized until slaughter, while flocks in the houses without boot-dipping became *Campylobacter* positive only 7-10 days after

hatching (Humphrey et al., 1993). In the Swedish study, the *Campylobacter* prevalence was 39% in houses lacking or having insufficient hygiene barriers, but in houses having strict hygiene barriers, the infection rate was only 22%; it also suggested that changing of boots before entering the house was effective in preventing *Campylobacter* colonization (Berndston et al., 1996).

Transmission among consecutive flocks has been suggested in certain studies, but is generally uncommon (Zweifel et al., 2008). Transmission of *Campylobacter* to subsequent flocks can be interrupted by efficient cleaning and disinfection before placement of a new flock. In the Swedish study, the *Campylobacter*-negative flocks often followed a positive flock, which indicated that normal cleaning and disinfecting routines were sufficient for eliminating *Campylobacter* from the house (Berndston et al., 1996). Several studies also suggested that there was cross-contamination of *Campylobacter* between houses by dust or air transmission, but this was not a predominant mode of transmission (Pearson et al., 1993).

Several studies have reported that the incidence of *Campylobacter* in broiler flocks is seasonal (Kapperud et al., 1993; Berndston et al., 1996). The Norwegian study demonstrated that *Campylobacter* colonization was significantly associated with geographic area and season: the flocks slaughtered in the autumn were more likely to be identified as *Campylobacter* contaminated (Kapperud et al., 1993). During 1992 to 1993, Jacobs-Reitsma et al. found that the *Campylobacter* contamination rate is lowest in March

in which only 50% of the flocks were contaminated by *Campylobacter*, but during the period June to September the *Campylobacter* contamination rate was extremely high and all the flocks tested were positive(Jacobs-Reitsma et al., 1994). However, the British study suggested that there is no apparent seasonal variation in *Campylobacter* colonization (Humphrey et al., 1993).

The Swedish study suggested that the broiler house structure may have influence on *Campylobacter* infection rate: the houses with floors made of wood were found with less *Campylobacter*-positive flocks than those with concrete or asphalt floors; ceilings made of wood or concrete were associated with less positive flocks than the less absorbing sheet-metal (Berndston et al., 1996). However, the British study demonstrated that there is no evidence that the prevalence of *Campylobacter* colonization was associated with broiler house floor structure: birds in house with concrete floors were as likely to be colonized with *Campylobacter* as those in house in houses with earth floors (Humphrey et al., 1993).

In conclusion, different findings were reported on transmission of *Campylobacter*, suggesting that *Campylobacter* transmission is very complex. This may be because numerous factors could influence the transmission of *Campylobacter*, such as environment difference between different farms, genetic distinction among *Campylobacter* and different practices on farms

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CHAPTER II:

Antimicrobial Susceptibility and Clonal Population Structure of Multidrug Resistant *Campylobacter jejuni* Isolates from Commercial Turkeys in North Carolina

2.1 Abstract

Campylobacter jejuni is the most frequently reported bacterial cause of human gastroenteritis in the industrialized world and contaminated poultry are a leading vehicle for this organism. Commercial turkey flocks in eastern North Carolina have been found to be colonized frequently with *C. jejuni* which are resistant to several antimicrobials (tetracycline, streptomycin, kanamycin, and nalidixic acid/ciprofloxacin). Such strains have been designated multidrug resistant (MDR). In this study, we assessed the prevalence of MDR *C. jejuni* in North Carolina between 2008 and 2012. Turkeys were from 467 flocks, operated by four different companies. Prevalence assessments suggest high prevalence of MDR *C. jejuni* in both brooders and grow-out turkeys. A total of 141 *C. jejuni* isolates were selected to be determined strain types by pulsed-field gel electrophoresis (PFGE) with SmaI. PFGE analysis revealed that MDR *C. jejuni* were genetically distinct from non-MDR *C. jejuni*. Bionumerics-based cluster analysis of PFGE profiles of MDR *C. jejuni* yielded three main clusters of isolates, of which one (cluster A2) included most of the MDR isolates. Multilocus sequence typing (MLST) of 5 MDR *C. jejuni* isolates representing all three clusters identified 2 sequence types (ST1839 and the closely related ST2936). The ST1839 was predominant and was detected among isolates from different farms. Analysis of MIC determinations revealed MDR *C. jejuni* were highly resistant to roxarsone, and were inhibited at relatively low concentrations of gentamicin and benzalkonium chloride.

2.2 Introduction

Campylobacter is a leading cause of human acute bacterial food-borne disease in the United States and other industrialized nations. In the United States alone, in spite of many undiagnosed or unreported cases, there is still an estimated 0.8 million *Campylobacter* infections every year, associated with an estimated 124 deaths (CDC, 2010). Reports by the Centers for Disease Control and Prevention (CDC) Foodborne Disease Surveillance Network (FoodNet) have shown that *Campylobacter* accounts for about 13 infections per 100,000 persons (Scallan et al., 2011).

Campylobacter is a zoonotic pathogen that can colonize the intestinal tracts of all food animals such as poultry, cattle and swine (D'Lima et al., 2007). *Campylobacter* infection is usually a self-limiting diarrheal disease, but can also have severe long-term sequelae such as reactive arthritis and Guillain–Barré syndrome (Humphrey et al., 2007). The most frequently identified sources of *Campylobacter* infections are untreated water, contaminated milk, meat, poultry and direct transmission from animals (Neimann et al., 2003). Poultry consumption (raw or undercooked poultry) and handling of poultry have been identified as major risk factors for human Campylobacteriosis (Altekruse et al., 2003). The majority (80% to 90%) of the *Campylobacter* infection cases involve *C. jejuni*, with most of the remainder being primarily due to *C. coli* (Friedman et al., 2000; Nachamkin et al., 2000). *C. jejuni* is more common among poultry and cattle than *C. coli* while *C. coli* is more commonly detected in swine (Aarestrup et al., 1997; Bae et al., 2005; Saenz et al., 2000).

Eastern North Carolina is a major contributor to turkey production in the United States. In a recent study, *C. jejuni* was found to the predominant species in commercial turkeys in this region (Wright et al, 2008). In the course of our laboratory's investigation of *Campylobacter* colonization in commercial turkeys, we have found high prevalence of *C. jejuni* isolates with multidrug-resistance phenotypes in eastern North Carolina (Gu et al., 2009). Gu et al. showed that the majority of the *C. jejuni* isolates (74%) were resistant to three or more antimicrobials (Gu et al., 2009).

Preliminary data from our laboratory indicated that *C. jejuni* of a certain antimicrobial susceptibility profile (TSKQ phonotype) were commonly and repeatedly isolated pre-harvest. This research characterized the strains that colonize turkeys in commercial farms from four companies (integrators) in Eastern North Carolina. PFGE and MLST methods were applied to determine the genetic diversity of isolates. We specifically looked into the strain prevalence of MDR *C. jejuni*. We investigated both genetic diversity among MDR *C. jejuni* and genetic distinction between MDR and non-MDR *C. jejuni*. We also looked into the level of resistance of the isolates to several antibiotics.

2.3 Materials and methods

***Campylobacter* strains, isolation and growth conditions.** The *C. jejuni* isolates used in this study are listed in Table 2.1. These isolates were selected from our laboratory *Campylobacter* culture collection and had been isolated between 2004 and 2012 from fecal or cecal samples from commercially grown turkeys in eastern North Carolina. The majority

of the isolates were from 2008-2012. Turkeys were from 114 farms operated by four different companies (integrators). To isolate *Campylobacter* from cecal or fecal samples, approximately 0.1 g of cecal contents from the incision of cecum or fecal samples from the interior of the dropping were directly streaked on to *Campylobacter* blood-free selective agar (CCDA; Oxoid, Hampshire, UK) with the corresponding supplement (SE 155, Oxoid) and incubated in anaerobic jars containing a CampyPak Plus microaerobic system (Becton Dickinson, Sparks, Md.) at 42℃ for 48 h. Bacteria were grown on Mueller Hinton agar (MHA) plates at 42℃ for 48 h under microaerobic conditions and preserved at -80℃ in 2ml tubes containing 1 ml of brain heart infusion (Difco, Sparks, MD) with 20% glycerol.

Antibiotic susceptibility and minimum inhibitory concentration (MIC) determinations. The isolates were screened for resistance to selected antibiotics (tetracycline (Sigma, St. Louis, MO), erythromycin (Sigma, St. Louis, MO), streptomycin (Sigma, St. Louis, MO), kanamycin (Fisher, Fair Lawn, NJ), nalidixic acids (Fisher, Fair Lawn, NJ) and ciprofloxacin (Fisher, Fair Lawn, NJ)) with the agar dilution method, following the Clinical Laboratory Standards Institute (CLSI) guidelines. *C. jejuni* ATCC 33560 (purchased from the American Type Culture Collection, Manassas, VA) was used as the quality control organism. Antibiotic resistance was determined by growth of bacteria following incubation at 42℃ for 48h at the following antibiotic concentrations: tetracycline (16 μg/ml), erythromycin (8 μg/ml), streptomycin (64 μg/ml), kanamycin (64 μg/ml), nalidixic acid (32 μg/ml) and ciprofloxacin (4 μg/ml). No growth of *C. jejuni* ATCC 33560 was observed for any of these antibiotics at

the indicated concentrations. Isolates were defined as resistant based on breakpoints of ≥16 μg/ml for tetracycline, ≥64 μg/ml for streptomycin, ≥64 μg/ml for kanamycin, ≥32 μg/ml for nalidixic acid and ≥4 μg/ml for ciprofloxacin.

MIC was defined as the lowest concentration of an antimicrobial agent that inhibited bacterial growth on the plates. MICs were determined for the following eleven antimicrobial agents: tetracycline, streptomycin, kanamycin, nalidixic acid, ciprofloxacin, gentamicin (Fisher, Fair Lawn, NJ), benzalkonium chloride (Acros, New Jersey), copper, cadmium (Sigma, St. Louis, MO), arsenic (Fluka, Steinheim, Germany) and roxarsone. The dilution range for tetracycline, streptomycin, kanamycin, nalidixic acid and ciprofloxacin was between 0.5 to 256 ug/ml; for gentamicin, between 0.5 to 2 ug /ml; for benzalkonium chloride, between 0.5 to 8 ug /ml; for copper, between 1 to 5 uM/ml; for cadmium, between 1 to 5 ug /ml; for arsenite, between 1 to 8 ug /ml; for arsenate, between 20 to 320 ug /ml; and for roxarsone, between 128 to 2048 ug /ml. MICs test were performed using the agar dilution method as described previously (Luangtongkum et al., 2006).

DNA extraction, species determination, PFGE and MLST. Genomic DNA was extracted from bacteria grown on MHA plates using a Qiagen DNeasy Tissue kit (Qiagen, Valencia, CA) as described previously (Smith et al., 2004). Species (*C. jejuni* or *C. coli*) was determined by multiplex PCR which employed the *C. jejuni*-specific *hip* primers and the *C. coli*-specific *ceu* primers as previously described (Smith et al., 2004). PFGE was performed using SmaI (New England Biolabs) according to the PulseNet protocol

(http://www.cdc.gov/PULSENET/protocols.htm), with some minor adjustment as previously described (D'Lima et al., 2007). Tagged Image File Format (TIFF) images of banding patterns resulting from PFGE were analyzed by BioNumerics (version 4.6, Applied Maths, Saint-Marten-Latem, Belgium). Grouping was done with the band position tolerance of Dice similarity coefficient at 1.5%, and clustering for genetic similarity was performed using the unweighted pair group method with arithmetic average (UPGMA), as previously described (D'Lima et al., 2007). For MLST, primer sets for seven housekeeping genes (*aspA, aptA, glnA, gltA, glyA, tkt and pgm*) were used, and PCR analysis was performed as previously described (Miller et al., 2006) with minor modifications (95 for 45s, 53 for 45s, and 72 for 2 min, over 32 cycles) as described (Gu et al., 2009). Amplicons were sequenced at Genwiz Laboratory (Genewiz, Inc., NJ). Sequence type (ST) identification was done using the *C. jejuni*/*C. coli* MLST database (http://pubmlst.org/*Campylobacter*).

MDR prevalence assessments. Two prevalence assessments methods were applied in this study: one assessed the frequency of MDR *C. jejuni* isolates among all *Campylobacter* isolates from four different companies between 2008 and 2012; the other assessed the frequency of flocks with one or more MDR *C. jejuni* isolates among all *Campylobacter*-positive flocks from four different companies between 2008 and 2012. Two way ANOVA analysis was used to determine if there is significant difference in MDR *C. jejuni* prevalence between different years and companies. Statistical analyses were conducted by SAS software (version 9.3, SAS Institute, Cary, NC).

2.4 Results

MDR *C. jejuni* was commonly encountered in turkeys from four different companies. In total, 1250 (57%) *Campylobacter* isolates were obtained from 2181 turkey cecal samples from turkeys (1-7 weeks of age) grown commercially in eastern North Carolina between January 2008 and May 2012. Turkeys were from 467 flocks operated by four different companies (integrators), with 106, 115, 104 and 142 flocks from company A, B, C and D, respectively. From these flocks, 264, 357, 279, and 350 of the *Campylobacter* isolates were obtained from company A, B, C and D, respectively. Of the 1250 *Campylobacter* isolates, 225 (18%) were identified as MDR *C. jejuni* in these five years, with a prevalence for different years ranging from 10% to 32% (Table 2.2 and Figure 2.1). A total of 467 young commercial turkey flocks from four different companies were investigated by our laboratory between January 2008 and May 2012. *Campylobacter* was isolated from one or more of the birds (ceca) of 325 (70%) of these flocks, with 71, 92, 69 and 93 flocks from company A, B, C and D, respectively. Of the 325 *Campylobacter*-positive flocks, 197 (61%) included one or more birds (ceca) colonized with MDR *C. jejuni*. The prevalence of MDR *C. jejuni*-positive flocks ranged from 25% to 41% in these years (Table 2.4 and Figure 2.2). Both isolates data and flocks data demonstrate that the prevalence of MDR *C. jejuni* fluctuated over time, with the lowest prevalence in 2009 and the highest prevalence in 2011 (Figure 2.1 and Figure 2.2). A significant increase ($P<0.05$) of MDR C. jejuni prevalence was found between 2009 and 2011, with a rapid increase in 2010 (Figure 2.1 and

Table 2.11). However, the prevalence dropped sharply in 2012, which resulted from the extremely low prevalence of MDR *C. jejuni* in company D in 2012.

The MDR *C. jejuni* prevalence varied from company to company, with 39 (15%), 77 (22%), 61 (22%) and 49 (12%) identified as MDR *C. jejuni* among company A, B, C and D respectively (Table 2.3). Between 2008 and 2012, a wide range of MDR *C. jejuni* prevalence was found among the four companies (6% to 44%, 10% to 55%, 8% to 56% and 3% to 42%, respectively) (Table 2.3 and Figure 2.3). We noted that all four companies had an increasing trend of prevalence from 2009 to 2010. Company A and C had a similar trend of MDR *C. jejuni* prevalence. These two companies had the same prevalence of MDR *C. jejuni* in both 2008 and 2009 and the prevalence reached its highest point in 2010. Interestingly, company B and company D also had a similar trend of MDR *C. jejuni* prevalence. The highest prevalence of MDR C. jejuni in these two companies was in 2011. There was a small difference in prevalence trends between company B and D: the MDR *C. jejuni* prevalence in company D kept increasing from 2008 to 2011, while the prevalence in company B decreased in 2009. The same result was also revealed by assessments of flock prevalence (Figure 2.4).

MDR *C. jejuni* was highly common in samples from brooders as well as grow-out birds. A total of 227 *Campylobacter* isolates were obtained from 260 turkey fecal samples from both brooder farms and grow-out farms between April 2012 and August 2012. Turkey fecal samples were from 5 brooder flocks and 14 grow-out flocks operated by two companies.

All 5 brooder flocks and 14 grow-out flocks were MDR *C. jejuni* positive, which indicated that MDR *C. jejuni* remained highly prevalent in these companies between April 2012 and August 2012. The prevalence of MDR *C. jejuni* isolates is shown in Table 2.6. We noted the total MDR *C. jejuni* prevalence (49%) in brooder samples was close to the total prevalence (55%) in samples from grow-out farms. Prevalence data show that MDR *C. jejuni* from fecal samples had much higher prevalence than those from cecal sample in the first half of 2012 (Table 2.2 and Table 2.6).

PFGE revealed 3 major clusters with 9 different PFGE profiles among MDR *C. jejuni*. A total of 141 *C. jejuni* isolates were typed with PFGE. The 141 isolates consist of seven different *C. jejuni* phenotypes. The majority (95/141) of the selected isolates were MDR *C. jejuni*, while the remainder were isolates which resistant to at most two of the six selected antibiotics (Table 2.1). SmaI profiles consist of 2 to 9 DNA fragments with sizes ranging from 48.5 – 1018.5 kb (Figure 2.5). The dendrogram of PFGE profiles from the 141 *C. jejuni* isolates is presented in Figure 2.6. The PFGE patterns between MDR and non-MDR *C. jejuni* are clearly distinct, suggesting that MDR and non-MDR *C. jejuni* had different genotypes. Except for isolates SC4177, 12470 and 12497, all PFGE patterns from MDR *C. jejuni* isolates had a clear band around 1000 kb; such as band was not observed for non-MDR isolates (Figure 2.6). Multiple weak bands were observed in the background between the major bands, suggesting partial digestion among MDR *C. jejuni*, which was not observed for non-MDR isolates (Figure 2.5 and Figure 2.6). We noted that only three MDR

C. jejuni isolates (12806, SC3400 and SC3416#1) did not have such evidence for partial digestion; all these three isolates had the same PFGE profile. In addition, this was the only PFGE profile among MDR *C. jejuni* that lacked the background putatively caused by DNase production.

As PFGE patterns with >95% similarity were considered identical, 9 different PFGE profiles were identified among 95 MDR *C. jejuni* isolates. Each PFGE profile was identified with a number from 1 to 9. The number of PFGE types is shown in Table 2.7. Of the 9 PFGE profiles, 4 were unique and 5 were shared by 2 to 68 isolates. The majority of the 95 MDR *C. jejuni* isolates were from company B and company C (39 and 48, respectively). As only a small number of MDR *C. jejuni* isolates were selected for PFGE from company A and D, they only have 3 and 1 PFGE profile types, respectively. We found that PFGE profile type 3 was shared by isolates from company A, B, C and D. PFGE profile type 1 was shared by isolates from company A, B and C. PFGE profile 4 and 5 were shared by isolates from company B and C. PFGE profile 9 was the only one shared by isolates from company A and C.

Based on > 80% similarity in PFGE profiles, we identified three major clusters (A1, A2 and A3) (Figure 2.2). Cluster A2 is the largest cluster with 70 isolates, followed by cluster A3 with 13 isolates and cluster A1 with 9 isolates. Each cluster involved 2 different PFGE profiles. PFGE profile type 6, 7 and 8 represented unique PFGE patterns. All these 3

unique PFGE profiles were especially different from others, which were more similar with PFGE patterns from non-MDR *C. jejuni* isolates than those from MDR *C. jejuni* isolates.

MLST identified 2 different STs among MDR *C. jejuni*; ST1839 was suggested as the most predominant MDR *C. jejuni* group. A total of 5 isolates were chosen among the 95 MDR *C. jejuni* for analysis by MLST. These 5 isolates represented the isolates from different PFGE-based clusters, with two from cluster A1, two from cluster A2 and one from cluster A3 (Figure 2.6 and Figure 2.7). In addition, four additional isolates (two of T phenotype and two of pan-sensitive phenotype) were included from the non-MDR panel. Two STs (ST2934 and ST353) were identified among the 4 non-MDR *C. jejuni* (Table 2.8). Two different STs were identified among the 5 MDR *C. jejuni* isolates (Table 2.8). Both ST1839 and ST2936 were turkey-specific STs. Both of these two STs were found only in turkey-derived *C. jejuni* in North Carolina before (*Campylobacter jejuni*/*coli* PubMLST database). MLST data suggest that ST1839 and ST2936 were genetically closely related; only one allele difference was observed among these two STs (Table 2.8).

Figure 2.7 demonstrates the difference in PFGE profiles among the 5 MDR isolates. Interestingly, although SC2225, SC2677 and SC2217 had clearly distinct PFGE patterns, especially for SC2677 which had <60% similarity with other PFGE patterns, they turned out to be the same ST. Since all 4 of the isolates were identified as ST1839, it appears that ST1839 was the most predominant MDR *C. jejuni* group colonizing the turkey flocks from company eastern North Carolina between 2008 and 2012.

MIC for antimicrobial agents. A total of 9 *C. jejuni* isolates were tested for MIC with gentamicin, benzalkonium chloride, copper, cadmium, arsenic and ROX. Of the 9 isolates, 5 were MDR *C. jejuni*, 2 were only resistant to tetracycline, and another 2 were pan-sensitive isolates which were sensitive to all six antimicrobials (table 2.9). All 9 isolates were inhibited at 8 ug/ml of arsenite, 2048 ug/ml of ROX, and relatively low concentrations of gentamicin and benzalkonium chloride. The 2 non-MDR isolates which were only resistant to tetracycline showed relatively low inhibitory concentration of both cadmium (1.0 ug/ml) and arsenate (80 ug/ml), while the others were inhibited at higher cadmium concentration (5.0 ug/ml) and higher arsenate concentration (4 isolates at 160 ug/ml and 3 isolates at 320 ug/ml). The majority of the isolates were inhibited at 2.0 uM/ml of copper, with only two isolates (SC2677 and SC4036) inhibited at 5.0 uM/ml. There was no noticeable or consistent difference in MICs between MDR and non-MDR *C. jejuni* isolates.

A total of 10 *C. jejuni* isolates were also tested for MICs to antibiotics to which the isolates were resistant. In the case of tetracycline, we identified only one isolates which was not inhibited at 256 ug/ml; the exception was SC2756 with TK phenotype. However, another TK phenotype isolate (SC2715) was inhibited at relatively low concentration (64 ug/ml). All of the 5 MDR *C. jejuni* isolates were inhibited at either 128 or 256 ug/ml of tetracycline. In the case of kanamycin, only one isolates (SC4297) was inhibited at 128

ug/ml. All other isolates exceeded the maximum of the tested range (>256ug/ml) (Table 2.10).

The majority of the MDR isolates were inhibited at 128 ug/ml of streptomycin. Only SC4152 was inhibited at 256 ug/ml. The non-MDR isolate (SC4040) with TS phenotype was the only one able to grow beyond 256 ug/ml of streptomycin.

The majority of the MDR isolates were inhibited at 64 ug/ml of nalidixic acid and 32 of ciprofloxacin. The non-MDR isolate (SC4150) with TQ phenotype showed relatively high resistant to both nalidixic acid (256 ug/ml) and ciprofloxacin (64 ug/ml). Interestingly, we noted that SC4170 was able to grow at higher nalidixic aid concentration but was inhibited at relatively low ciprofloxacin concentration (16 ug/ml).

2.5 Discussion

In this study, we used two prevalence assessing methods to analyze prevalence of MDR *C. jejuni* in young conventionally grown turkeys from eastern North Carolina between 2008 and 2012; one method assessed prevalence of total MDR *C. jejuni* isolates, while the other one assessed prevalence of flocks with at least one MDR *C. jejuni* isolates. Both methods revealed that MDR *C. jejuni* had the lowest prevalence in 2009 and highest prevalence in 2010. There was a significant increase ($p<0.05$) of MDR *C. jejuni* prevalence between 2009 and 2011. Although the prevalence trends revealed by these two assessing methods were similar, the prevalence data obtained by assessing positive flocks were much steadier than those obtained from prevalence of isolates. This may be because prevalence based on

total isolates is more susceptible to fluctuations depending on number of isolates, or age of the bird.

The MDR *C. jejuni* prevalence data collected from fecal samples between April 2012 and August 2012 demonstrated that MDR *C. jejuni* was similarly prevalent both in young and old turkeys. Compared to the prevalence data collected from cecal samples in the first half of 2012, MDR *C. jejuni* had higher prevalence among fecal samples. As *Campylobacter* mainly colonizes the cecum, it is generally assumed that cecal samples often have a higher number of *Campylobacter* per gram than fecal samples (Rudi et al., 2004). However, *Campylobacter* strains found along the GI tract may differ depending on site. Preliminary data from our laboratory indicate that it is more likely to find *C. coli* in the cecum, and *C. jejuni* in the jejunum (D. Carver, R. M. Siletzky and S. Kathariou, unpublished findings). Thus, fecal samples are more likely to represent what present in GI tract than those from cecal samples.

MLST analysis of MDR *C. jejuni* isolates revealed 2 STs in this study. Both ST1839 and ST2936 were previously only reported in turkey isolates from North Carolina (*Campylobacter jejuni*/*coli* PubMLST database). A single ST (ST1839) was identified from all four representative isolates from three major PFGE-based clusters, which indicated that ST1839 was the most predominant MDR *C. jejuni* group among different turkey farms in eastern North Carolina. Previous studies in our laboratory yielded a similar result in 2008 (Gu et al., 2009). However, compared to this previous study which found three

predominant STs (ST1839, ST2132 and ST1162) among 13 MDR *C. jejuni* isolates, our study suggested that there might be a decrease in diversity of sequence types in MDR C. jejuni between 2008 and 2012. Interestingly, that ST2936 was also found in our laboratory, but, with a different phenotype (TSQ) (Gu et al., 2009). This implicates that there were phenotype diversity among ST2936 or ST2936 developed kanamycin resistance among these years. MLST analysis of non-MDR *C. jejuni* isolates revealed 2 STs, with three of four are ST2934 and one is ST353. ST2934 was found previously in our laboratory, while ST353 was not.

MDR *C. jejuni* isolates tended to be found in the same cluster on the dendrogram constructed by PFGE data, which indicated that isolates with the same antimicrobial resistance profiles tented to have closely related genotypes. A significant difference was found in PFGE profiles between MDR and non-MDR *C. jejuni*; specifically, PFGE patterns from MDR *C. jejuni* isolates had a clear band around 1000 kb, which differed from those PFGE patterns from sensitive *C. jejuni* isolates. The MLST result demonstrated a similar finding. The most MDR *C. jejuni* isolates investigated in this study were believed to have the same ST or closely relates STs: ST2936 was closely related to ST1839, with only one allele difference (Table 2.8). The majority of the MDR *C. jejuni* isolates exhibited partial digestion. The same result was observed in the previous studies in out laboratory (Gu et al., 2009). It can be hypothesized that SmaI did not cut all restriction sites completely, possibly because MDR *C. jejuni* had DNA that was partially methylated at SmaI sites.

Our study has shown the importance of combined use of genotyping methods to characterize MDR *C. jejuni*. PFGE can provide more discriminatory power than MLST, which can identify distinct subtypes among isolates with the same ST.

MIC determinations revealed that all tested *C. jejuni* including MDR *C. jejuni* were highly resistant to roxarsone. Roxarsone has been widely used as a feed additive in poultry production in the United States and other countries (Chapman and Johnson, 2002), and roxarsone will also convert to arsenite and arsenate (Cortinas et al., 2006). As *C. jejuni* colonizes the intestinal tracts of poultry, it is likely that *C. jejuni* are exposed to arsenic and build resistance. Mechanisms for arsenic resistance in *C. jejuni* have been characterized (Wang et al., 2009). A study by Sapkota et al. suggested that *Campylobacter* isolates from conventional poultry products were significantly more resistant to roxarsone than those from antimicrobial-free poultry products (Sapkota et al., 2006). We also observed that MDR *C. jejuni* had a certain extent of resistance to both arsenite and arsenate.

We also evaluated the antibacterial activity of copper against *C. jejuni.* The majority of MDR *C. jejuni* were inhibited at 2.0 uM/ml of $CuSO_4$. High concentration of Cu has a toxic effect on most microorganisms, with blocking of functional groups of proteins, inactivation of enzymes and alterations of membrane integrity (Mead et al., 1999).

We determined the levels of antibiotics resistance to which MDR *C. jejuni* isolates were resistant. All 5 MDR *C. jejuni* isolates showed high resistant to both tetracycline and kanamycin which is consistent with findings reported by our laboratory (Gu et al., 2009),

that there was a strong correlation between kanamycin resistance and tetracycline resistance. Nalidixic acid and ciprofloxacin are both fluoroquinolones and have similar mechanisms to inhibit *C. jejuni*. Although fluoroquinolones have been banned in poultry industry for several years, our finding still demonstrated high levels of resistance to both nalidixic acid and ciprofloxacin.

In conclusion, with the combined use of genotyping methods, we have provided evidence for the existence of a prevalent clonal group of MDR *C. jejuni* colonizing commercial grown turkeys in eastern North Carolina. We have shown the prevalence of MDR *C. jejuni* among different farms between 2008 and 2012. The results from our genotyping could be used as reference for further investigation to determine the reason why such clonal groups were so prevalent. Further studies are needed to identify and characterize the possible routes of transmission of these clonal groups of MDR *C. jejuni* in turkey production.

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Table 2.1. List of *C. jejuni* isolates investigated in this study.

ID	Received	Source	Company Code[b]	Farm code	Age	MLST	ARP[a]
SC681	2004/1/21	Cecal	B	40	49d		TSKQ
SC778	2004/1/22	Cecal	C	76	47d		TK
SC931	2004/7/14	Cecal	A	11	45d		TS
SC964	2004/7/14	Cecal	B	31	42d		TSKQ
SC1648	2007/3/7	Cecal	B	39	44d		TSKQ
SC1789-3	2007/6/27	Cecal	A	10	43d		TSKQ
SC1850	2007/6/27	Cecal	B	25	14d		TSKQ
SC2045	2008/1/16	Cecal	B	37	35 d		T
SC2065	2008/1/16	Cecal	B	17	41 d		TSKQ
SC2037	2008/1/16	Cecal	B	33	21 d		TSKQ
SC2023	2008/1/16	Cecal	A	9	12 d		TSKQ
SC2088	2008/1/17	Cecal	C	74	35 d		TSKQ
SC2226	2008/5/21	Cecal	B	23	39 d		TK
SC2205	2008/5/21	Cecal	B	17	42 d		TSKQ
SC2225	2008/5/21	Cecal	B	23	39 d	1839	TSKQ
SC2222	2008/5/21	Cecal	B	21	40 d		TSKQ
SC2209	2008/5/21	Cecal	B	18	40 d		TSKQ
SC2214	2008/5/21	Cecal	B	29	36 d		TSKQ
SC2217	2008/5/21	Cecal	B	35	39 d	1839	TSKQ
SC2272	2008/5/22	Cecal	C	72	33 d		T
SC2276	2008/5/22	Cecal	C	65	23 d		T
SC2264	2008/5/22	Cecal	C	85	21 d		TK
SC2277	2008/5/22	Cecal	C	65	23 d		TSKQ
SC2484	2008/12/4	Cecal	C	78	38 d		TSKQ
SC2677	2009/6/17	Cecal	B	24	46 d	1839	TSKQ
SC2674	2009/6/17	Cecal	B	42	39 d		TSKQ
SC2745	2009/6/25	Cecal	C	86	21 d	2934	-
SC2715	2009/6/25	Cecal	C	83	37 d		TK
SC2727	2009/6/25	Cecal	C	66	40 d		TK
SC2756	2009/6/25	Cecal	C	87	33 d		TK
SC2746	2009/6/25	Cecal	C	86	21 d		TSKQ
SC2934	2009/12/2	Cecal	B	41	35 d		T
SC2906	2009/12/2	Cecal	B	27	57 d		TSKQ
SC2920	2009/12/2	Cecal	B	38	21 d		TSKQ
SC2925	2009/12/2	Cecal	B	34	39 d		TSKQ
SC2950	2009/12/3	Cecal	C	75	42 d		T

Table 2.1. Continued.

SC2950	2009/12/3	Cecal	C	75	42 d		T
SC2988	2009/12/3	Cecal	C	64	28 d		T
SC2971	2009/12/3	Cecal	C	67	28 d		TK
SC2982	2009/12/3	Cecal	C	77	35 d		TK
SC2965	2009/12/3	Cecal	C	87	23 d		TSKQ
SC3176	2010/2/17	Cecal	B	22	49 d		T
SC3154	2010/2/17	Cecal	B	30	42 d		TSKQ
SC3404	2010/5/12	Cecal	B	36	41 d		T
SC3400	2010/5/12	Cecal	B	26	37 d		TSKQ
SC3416/#1	2010/5/12	Cecal	B	20	48 d		TSKQ
SC3435-1/#1	2010/5/13	Cecal	C	71	28 d	353	-
SC3442/#1	2010/5/13	Cecal	C	82	30 d		T
SC3449	2010/5/13	Cecal	C	84	40 d		TK
SC3543/#1	2010/8/18	Cecal	B	32	47 d		Q
SC3558/#1	2010/8/18	Cecal	B	43	54 d		T
SC3590	2010/8/19	Cecal	C	80	35 d		TSKQ
SC3596	2010/8/19	Cecal	C	68	44 d		TSKQ
SC3608	2010/8/19	Cecal	C	70	44 d		TSKQ
SC3578	2010/8/19	Cecal	C	81	26 d		TSKQ
SC3712/#1	2011/1/19	Cecal	B	50	45 d		T
SC3736/#1	2011/1/19	Cecal	B	58	27 d		TSKQ
SC3699/#1	2011/1/19	Cecal	B	60	53 d		TSKQ
SC3725/#1	2011/1/19	Cecal	B	51	32 d		TSKQ
SC3745	2011/1/20	Cecal	C	88	31 d		T
SC3764	2011/1/20	Cecal	C	93	40 d		T
SC3765	2011/1/20	Cecal	C	93	40 d		TSKQ
SC3881	2011/8/17	Cecal	B	57	26 d		T
SC3841	2011/8/17	Cecal	C	91	36 d		T
SC3873	2011/8/17	Cecal	C	102	19 d		T
SC3844	2011/8/17	Cecal	C	91	36 d		TK
SC3843	2011/8/17	Cecal	C	91	36 d		TQ
SC3875	2011/8/17	Cecal	C	102	19 d		TSKQ
SC3909	2011/8/17	Cecal	B	56	39 d		TSKQ
SC3886	2011/8/17	Cecal	B	53	43 d		TSKQ
SC3851	2011/8/17	Cecal	C	97	26 d		TSKQ
SC3895	2011/8/17	Cecal	B	61	22 d		TSKQ
SC3879	2011/8/17	Cecal	B	57	26 d		TSKQ
SC3902	2011/8/17	Cecal	B	52	29 d		TSKQ

Table 2.1. Continued.

SC3837	2011/8/17	Cecal	C	96	22 d		TSKQ
SC3860	2011/8/17	Cecal	C	92	21 d		TSKQ
SC4019	2011/12/15	Cecal	A	16	13 d	2934	T
SC4036	2011/12/15	Cecal	A	7	21 d	2934	T
SC4030	2011/12/15	Cecal	A	2	35 d		T
SC4042	2011/12/15	Cecal	A	14	16 d		T
SC4040	2011/12/15	Cecal	A	14	16 d		TS
SC4021	2011/12/15	Cecal	A	16	13 d		TSKQ
SC4025	2011/12/15	Cecal	A	12	13 d		TSKQ
SC4033	2011/12/15	Cecal	A	1	35 d		TSKQ
SC4065	2012/2/21	Cecal	A	8	28 d		TSKQ
SC4068	2012/2/21	Cecal	A	6	24 d		TSKQ
SC4150	2012/2/22	Cecal	B	49	37 d		TQ
SC4177	2012/2/22	Cecal	B	63	29 d		TSKQ
SC4152	2012/2/22	Cecal	B	59	25 d	1839	TSKQ
SC4100	2012/2/22	Cecal	C	89	28 d		TSKQ
SC4110	2012/2/22	Cecal	C	99	29 d		TSKQ
SC4158	2012/2/22	Cecal	B	54	34 d		TSKQ
SC4161	2012/2/22	Cecal	B	55	33 d		TSKQ
SC4115	2012/2/22	Cecal	C	100	29 d		TSKQ
SC4117	2012/2/22	Cecal	C	101	36 d		TSKQ
SC4134	2012/2/22	Cecal	C	98	33 d		TSKQ
SC4146	2012/2/22	Cecal	B	49	37 d		TSKQ
SC4144	2012/2/22	Cecal	C	95	21 d		TSKQ
SC4128	2012/2/22	Cecal	C	94	26 d		TSKQ
SC4104	2012/2/22	Cecal	C	90	27 d		TSKQ
SC4170	2012/2/22	Cecal	B	62	32 d	2936	TSKQ
12474	2012/4/23	Fecal	B	137	21 week		TK
12464	2012/4/23	Fecal	B	135	21 week		TSKQ
12470	2012/4/23	Fecal	B	136	21 week		TSKQ
SC4221	2012/5/15	Cecal	D	123	27 d		T
SC4218	2012/5/15	Cecal	D	123	27 d		TK
SC4250	2012/5/15	Cecal	D	125	24 d		TSKQ
SC4307	2012/5/16	Cecal	B	46	31 d		T
SC4297	2012/5/16	Cecal	B	48	25 d		TK
SC4270	2012/5/16	Cecal	C	112	18 d		TSKQ
SC4356	2012/5/17	Cecal	A	5	21 d		TQ
12485	2012/5/29	Fecal	B	138	123 d		TSKQ

Table 2.1. Continued.

12492	2012/6/6	Fecal	C	106	137 d		TK
12501	2012/6/6	Fecal	C	117	137 d		TSKQ
12497	2012/6/6	Fecal	C	106	137 d		TSKQ
12580	2012/7/6	Fecal	C	114	72 d		TK
12585	2012/7/6	Fecal	C	107	72 d		TSKQ
12552	2012/7/6	Fecal	C	109	72 d		TSKQ
12574	2012/7/6	Fecal	C	114	72 d		TSKQ
12562	2012/7/6	Fecal	C	118	69 d		TSKQ
12608	2012/7/10	Fecal	C	108	81 d		TSKQ
12620	2012/7/10	Fecal	C	113	73 d		TSKQ
12595	2012/7/10	Fecal	C	116	81 d		TSKQ
12657	2012/7/16	Fecal	C	111	26 d		TSKQ
12686	2012/7/17	Fecal	C	115	80 d		TSKQ
12710	2012/7/17	Fecal	C	105	83 d		TSKQ
12670	2012/7/17	Fecal	C	110	88 d		TSKQ
12806	2012/7/25	Fecal	C	104	28 d		TSKQ
12815	2012/7/27	Fecal	C	112	28 d		TSKQ
12827	2012/7/30	Fecal	B	45	35 d		TSKQ
12841	2012/8/9	Fecal	B	47	30 d		TSKQ
12877	2012/8/14	Fecal	C	110	116 d		TSKQ
12912	2012/8/29	Fecal	C	116	131 d		TSKQ
12886	2012/8/29	Fecal	C	103	131 d		TSKQ
12900	2012/8/29	Fecal	C	108	131 d		TSKQ
12941	2012/9/6	Fecal	C	107	134 d		TQ
12950	2012/9/6	Fecal	C	114	134 d		TSKQ
12939	2012/9/6	Fecal	C	107	134 d		TSKQ
12997	2012/9/6	Fecal	C	109	134 d		TSKQ
12925	2012/9/6	Fecal	C	116	21 week		TSKQ
12965	2012/9/6	Fecal	C	105	134 d		TSKQ
12984	2012/9/6	Fecal	C	118	138 d		TSKQ

[a] ARP – antimicrobial resistant profile (T – tetracycline, S – streptomycin, K – kanamycin, and Q - quinolones)

[b]After 2010, company B and company C merged.

Table 2.2. MDR *C. jejuni* prevalence in young turkeys by year (isolates).

Year	Company	No. of samples	No. of *Campylobacter*	No. of *C. jejuni*	No. of MDR *C. jejuni*	MDR *C. jejuni* / *Campylobacter*[a]	MDR *C. jejuni* / *C. jejuni*[b]
2008	A	105	69	28	11	16%	39%
	B	124	94	33	20	21%	61%
	C	135	69	33	11	16%	33%
	D	192	145	22	10	7%	45%
	Total	556	377	116	52	14%	45%
2009	A	81	65	23	5	8%	22%
	B	94	94	10	9	10%	90%
	C	102	65	30	5	8%	17%
	D	123	70	29	11	16%	38%
	Total	400	294	92	30	10%	33%
2010	A	80	34	24	15	44%	63%
	B	129	74	29	12	16%	41%
	C	143	25	22	14	56%	64%
	D	209	48	29	10	21%	34%
	Total	561	181	104	51	28%	49%
2011	A	77	36	12	2	6%	17%
	B	81	40	29	22	55%	76%
	C	89	39	29	10	26%	34%
	D	81	36	16	15	42%	94%
	Total	328	151	86	49	32%	57%
2012	A	100	60	9	6	10%	67%
	B	74	55	24	14	25%	58%
	C	104	81	25	21	26%	84%
	D	58	51	5	2	4%	40%
	Total	336	247	63	43	17%	68%

[a] Percentage of number of MDR *C. jejuni* to total number of *Campylobacter*

[b] Percentage of number of MDR *C. jejuni* to total number of *C. jejuni*

Table 2.3. MDR *C. jejuni* prevalence in young turkeys by company (isolates).

Company	Year	No. of samples	No. of *Campylobacter*	No. of *C. jejuni*	No. of MDR *C. jejuni*	MDR *C. jejuni* / *Campylobacter*[a]	MDR *C. jejuni* / *C. jejuni*[b]
A	2008	105	69	28	11	16%	39%
	2009	81	65	23	5	8%	22%
	2010	80	34	24	15	44%	63%
	2011	77	36	12	2	6%	17%
	2012	100	60	9	6	10%	67%
	Total	443	264	96	39	15%	41%
B	2008	124	94	33	20	21%	61%
	2009	94	94	10	9	10%	90%
	2010	129	74	29	12	16%	41%
	2011	81	40	29	22	55%	76%
	2012	74	55	24	14	25%	58%
	Total	502	357	125	77	22%	62%
C	2008	135	69	33	11	16%	33%
	2009	102	65	30	5	8%	17%
	2010	143	25	22	14	56%	64%
	2011	89	39	29	10	26%	34%
	2012	104	81	25	21	26%	84%
	Total	573	279	139	61	22%	44%
D	2008	192	145	22	10	7%	45%
	2009	123	70	29	11	16%	38%
	2010	209	48	29	10	21%	34%
	2011	81	36	16	15	42%	94%
	2012	58	51	5	2	4%	40%
	Total	663	350	101	48	14%	47%

[a] Percentage of number of MDR *C. jejuni* to total number of *Campylobacter*

[b] Percentage of number of MDR *C. jejuni* to total number of *C. jejuni*

Table 2.4. MDR *C. jejuni* prevalence in young turkeys by year (flocks).

Year	Company	No. of total flocks	No. of *Campy*-positive flocks	No. of *C. jejuni*-positive flocks	No. of MDR *C. jejuni*-positive flocks	MDR *C. jejuni* / *Campylobacter*[a]	MDR *C. jejuni* / *C. jejuni*[b]
2008	A	26	21	13	7	33%	54%
	B	30	25	16	11	44%	69%
	C	24	16	11	6	38%	55%
	D	43	36	10	6	17%	60%
	Total	123	98	50	30	31%	60%
2009	A	13	12	8	4	33%	50%
	B	34	28	9	6	21%	67%
	C	17	13	11	3	23%	27%
	D	25	16	8	4	25%	50%
	Total	89	69	36	17	25%	47%
2010	A	20	8	7	6	75%	86%
	B	20	16	9	3	19%	33%
	C	24	9	9	5	56%	56%
	D	36	11	8	3	27%	38%
	Total	100	44	33	17	39%	52%
2011	A	29	18	12	6	33%	50%
	B	16	11	10	8	73%	80%
	C	15	10	9	5	50%	56%
	D	27	19	10	5	26%	50%
	Total	87	58	41	24	41%	59%
2012	A	18	12	5	2	17%	40%
	B	15	12	8	6	50%	75%
	C	24	21	10	10	48%	100%
	D	11	11	3	2	18%	67%
	Total	68	56	26	20	36%	77%

[a] Percentage of number of MDR *C. jejuni* positive flocks to total number of *Campylobacter* positive flocks

[b] Percentage of number of MDR *C. jejuni* positive flocks to total number of *C. jejuni* positive flocks

Table 2.5. MDR *C. jejuni* prevalence in young turkeys by company (flocks).

Company	Year	No. of total flocks	No. of *Camp*-positive flocks	No. of *C. jejuni* positive flocks	No. of MDR *C. jejuni* positive flocks	MDR *C. jejuni* / *Campylobacter*[a]	MDR *C. jejuni* / *C. jejuni*[b]
A	2008	26	21	13	7	33%	54%
	2009	13	12	8	4	33%	50%
	2010	20	8	7	6	75%	86%
	2011	29	18	12	6	33%	50%
	2012	18	12	5	2	17%	40%
	Total	106	71	45	25	35%	56%
B	2008	30	25	16	11	44%	69%
	2009	34	28	9	6	21%	67%
	2010	20	16	9	3	19%	33%
	2011	16	11	10	8	73%	80%
	2012	15	12	8	6	50%	75%
	Total	115	92	52	34	37%	65%
C	2008	24	16	11	6	38%	55%
	2009	17	13	11	3	23%	27%
	2010	24	9	9	5	56%	56%
	2011	15	10	9	5	50%	56%
	2012	24	21	10	10	48%	100%
	Total	104	69	50	29	42%	58%
D	2008	43	36	10	6	17%	60%
	2009	25	16	8	4	25%	50%
	2010	36	11	8	3	27%	38%
	2011	27	19	10	5	26%	50%
	2012	11	11	3	2	18%	67%
	Total	142	93	39	20	22%	51%

[a] Percentage of number of MDR *C. jejuni* positive flocks to total number of *Campylobacter* positive flocks

[b] Percentage of number of MDR *C. jejuni* positive flocks to total number of *C. jejuni* positive flocks

Table 2.6. MDR *C. jejuni* prevalence in brooder farms and grow-out farms (isolates).

	Flock code	No. of samples	No. of *Campylobacter*	No. of *C. jejuni*	No. of MDR *C. jejuni*	MDR *C. jejuni* /*Campylobacter*[a]	MDR *C. jejuni* / *C. jejuni*[b]
Brooder farms	B1	12	11	3	3	27%	100%
	B3	12	12	6	3	25%	50%
	B4	12	10	10	10	100%	100%
	B5	12	11	4	4	36%	100%
	B6	12	7	7	5	71%	71%
	Total	60	51	30	25	49%	83%
Grow out farms	B1.1	12	12	4	2	17%	50%
	B1.2	12	12	9	9	75%	100%
	B1.3	12	12	10	9	75%	90%
	B1.4	12	11	8	5	45%	63%
	B2.1	12	12	9	9	75%	100%
	B2.2	12	12	9	7	58%	78%
	B3.1	12	12	3	3	25%	100%
	B3.2	12	12	9	8	67%	89%
	B3.3	12	12	7	7	58%	100%
	B3.4	12	10	6	6	60%	100%
	B4.1	12	12	9	9	75%	100%
	B4.2	12	12	5	2	17%	40%
	B4.3	24	23	16	12	52%	75%
	B4.4	12	12	10	8	67%	80%
	Total	180	176	114	96	55%	84%

1 Young turkeys from brooder farm B1 were delivered into grow out farms B1.1, B1.2 B1.3 and B1.4; Young turkeys from brooder farm B3 were delivered into grow out farms B3.1, B.3.2, B.3.3 and B3.4; Young turkeys from brooder farm B4 were delivered into grow out farms B4.1, B4.2, B4.3 and B4.4.

[a] Percentage of number of MDR *C. jejuni* to total number of *Campylobacter*

[b] Percentage of number of MDR *C. jejuni* to total number of *C. jejuni*

Table 2.7. Source and PFGE type of the MDR *C. jejuni* isolates from 4 companies.

ID	Received	Company	Farm	Age	PFGE type
SC681[a]	2004/1/21	B	40	49d	3
SC964[a]	2004/7/14	B	31	42d	3
SC1648[a]	2007/3/7	B	39	44d	3
SC1850[a]	2007/6/27	B	25	14d	1
SC1789-3[a]	2007/6/27	A	10	43d	9
SC2023	2008/1/16	A	9	12 d	1
SC2065	2008/1/16	B	17	41 d	3
SC2037	2008/1/16	B	33	21 d	3
SC2088	2008/1/17	C	74	35 d	1
SC2209	2008/5/21	B	18	40 d	1
SC2214	2008/5/21	B	29	36 d	1
SC2217	2008/5/21	B	35	39 d	1
SC2205	2008/5/21	B	17	42 d	3
SC2225	2008/5/21	B	23	39 d	3
SC2222	2008/5/21	B	21	40 d	3
SC2277	2008/5/22	C	65	23 d	3
SC2484	2008/12/4	C	78	38 d	2
SC2674	2009/6/17	B	42	39 d	3
SC2677	2009/6/17	B	24	46 d	5
SC2746	2009/6/25	C	86	21 d	5
SC2906	2009/12/2	B	27	57 d	3
SC2920	2009/12/2	B	38	21 d	3
SC2925	2009/12/2	B	34	39 d	3
SC2965	2009/12/3	C	87	23 d	1
SC3154	2010/2/17	B	30	42 d	3
SC3400	2010/5/12	B	26	37 d	5
SC3416/#1	2010/5/12	B	20	48 d	5
SC3596	2010/8/19	C	68	44 d	3
SC3608	2010/8/19	C	70	44 d	3
SC3578	2010/8/19	C	81	26 d	3
SC3590	2010/8/19	C	80	35 d	5
SC3699/#1	2011/1/19	B	60	53 d	3
SC3725/#1	2011/1/19	B	51	32 d	3
SC3736/#1	2011/1/19	B	58	27 d	5
SC3765	2011/1/20	C	93	40 d	3
SC3886	2011/8/17	B	53	43 d	3
SC3851	2011/8/17	C	97	26 d	3

Table 2.7. Continued.

SC3895	2011/8/17	B	61	22 d	3
SC3879	2011/8/17	B	57	26 d	3
SC3902	2011/8/17	B	52	29 d	3
SC3837	2011/8/17	C	96	22 d	3
SC3860	2011/8/17	C	92	21 d	3
SC3875	2011/8/17	C	102	19 d	5
SC3909	2011/8/17	B	56	39 d	5
SC4021	2011/12/15	A	16	13 d	3
SC4025	2011/12/15	A	12	13 d	3
SC4033	2011/12/15	A	1	35 d	3
SC4065	2012/2/21	A	8	28 d	3
SC4068	2012/2/21	A	6	24 d	3
SC4170	2012/2/22	B	62	32 d	1
SC4152	2012/2/22	B	59	25 d	3
SC4100	2012/2/22	C	89	28 d	3
SC4110	2012/2/22	C	99	29 d	3
SC4158	2012/2/22	B	54	34 d	3
SC4161	2012/2/22	B	55	33 d	3
SC4115	2012/2/22	C	100	29 d	3
SC4117	2012/2/22	C	101	36 d	3
SC4134	2012/2/22	C	98	33 d	3
SC4146	2012/2/22	B	49	37 d	3
SC4144	2012/2/22	C	95	21 d	3
SC4128	2012/2/22	C	94	26 d	3
SC4104	2012/2/22	C	90	27 d	3
SC4177	2012/2/22	B	63	29 d	6
12464	2012/4/23	B	135	21 week	4
12470	2012/4/23	B	136	21 week	7
SC4250	2012/5/15	D	125	24 d	3
SC4270	2012/5/16	C	112	18 d	4
12485	2012/5/29	B	138	123 d	3
12501	2012/6/6	C	117	137 d	9
12497	2012/6/6	C	106	137 d	8
12552	2012/7/6	C	109	72 d	3
12574	2012/7/6	C	114	72 d	3
12562	2012/7/6	C	118	69 d	3
12585	2012/7/6	C	107	72 d	5
12608	2012/7/10	C	108	81 d	3

Table 2.7. Continued.

12620	2012/7/10	C	113	73 d	3
12595	2012/7/10	C	116	81 d	3
12657	2012/7/16	C	111	26 d	3
12686	2012/7/17	C	115	80 d	3
12710	2012/7/17	C	105	83 d	3
12670	2012/7/17	C	110	88 d	3
12806	2012/7/25	C	104	28 d	5
12815	2012/7/27	C	112	28 d	3
12827	2012/7/30	B	45	35 d	3
12841	2012/8/9	B	47	30 d	3
12877	2012/8/14	C	110	116 d	3
12886	2012/8/29	C	103	131 d	3
12900	2012/8/29	C	108	131 d	3
12912	2012/8/29	C	116	131 d	5
12950	2012/9/6	C	114	134 d	3
12939	2012/9/6	C	107	134 d	3
12997	2012/9/6	C	109	134 d	3
12925	2012/9/6	C	116	21 week	3
12965	2012/9/6	C	105	134 d	3
12984	2012/9/6	C	118	138 d	3

[a] Five isolates before 2008 were included in this study to investigate genetic similarity of MDR *C. jejuni* among years.

Table 2.8. MLST alleles and sequence type (STs) of 9 *C. jejuni* isolates.

	aspA	glnA	gltA	glyA	pgm	tkt	atpA	APR [a]	ST
SC2217	2	222	29	250	303	25	35	TSKQ	ST1839
SC2225	2	222	29	250	303	25	35	TSKQ	ST1839
SC2677	2	222	29	250	303	25	35	TSKQ	ST1839
SC4152	2	222	29	250	303	25	35	TSKQ	ST1839
SC4170	2	2	29	250	303	25	35	TSKQ	ST2936
SC4036	7	112	5	2	167	67	6	T	ST2934
SC4019	7	112	5	2	167	67	6	T	ST2934
SC2745	7	112	5	2	167	67	6	T	ST2934
SC3435-1	7	17	5	2	10	3	6	Pan-sensitive	ST353

[a] ARP – antimicrobial resistant profile (T – tetracycline, S – streptomycin, K – kanamycin, and Q - quinolones)

Table 2.9. MICs for selected *C. jejuni* isolates from turkeys between 2008 and 2012.

Strain	AB	MICs [a]						
		Arsenite ug/ml	BC ug/ml	ROX ug/ml	G ug/ml	$CuSO_4$ uM/ml	Cd ug/ml	Arsenate ug/ml
SC2677	TSKQ	8.0	0.5	2048	1.0	5.0	5.0	160
SC4152	TSKQ	8.0	0.5	2048	1.0	2.0	5.0	160
SC4170	TSKQ	8.0	0.5	2048	1.0	2.0	5.0	160
SC2217	TSKQ	8.0	1.0	2048	2.0	2.0	5.0	320
SC2225	TSKQ	8.0	1.0	2048	1.0	2.0	5.0	320
SC4036	T	8.0	1.0	2048	1.0	5.0	1.0	80
SC4019	T	8.0	0.5	2048	1.0	2.0	1.0	80
SC2745	-	8.0	1.0	2048	1.0	2.0	5.0	160
SC3435-1	-	8.0	1.0	2048	1.0	2.0	5.0	320

[a] BC –benzalkonium chloride, ROX –roxarsone, G –gentamicin and Cd - cadmium

Table 2.10. Antibiotic MICs for selected *C. jejuni* isolates from turkeys between 2008 and 2012.

Strain	AB	MICs [a]				
		Tetra ug/ml	Stre ug/ml	Kana ug/ml	Nali ug/ml	Cipro ug /ml
SC2677	TSKQ	128	128	>256	64	32
SC4152	TSKQ	256	256	>256	64	32
SC4170	TSKQ	256	128	>256	128	16
SC2217	TSKQ	128	128	>256	64	64
SC2225	TSKQ	256	128	>256	64	32
SC2756	TK	>256	-	>256	-	-
SC2715	TK	64	-	>256	-	-
SC4297	TK	>256	-	128	-	-
SC4040	TS	256	>256	-	-	-
SC4150	TQ	256	-	-	256	64

[a] Tetra - tetracycline, Stre - streptomycin, Kana - kanamycin, Nali - nalidixic acid and Cipro- ciprofloxacin

Table 2.11. Pairwise comparison of MDR *C. jejuni* prevalence between years using LSD.

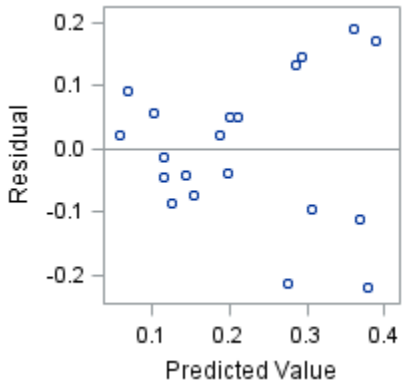
The SAS System

The GLM Procedure
t Tests (LSD) for percentage

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	0.019488
Critical Value of t	2.13145
Least Significant Difference	0.2104

Means with the same letter are not significantly different.

t Grouping		Mean	N	year
	A	0.34250	4	2010
	A			
	A	0.32250	4	2011
	A			
B	A	0.16250	4	2012
B	A			
B	A	0.15000	4	2008
B				
B		0.10500	4	2009



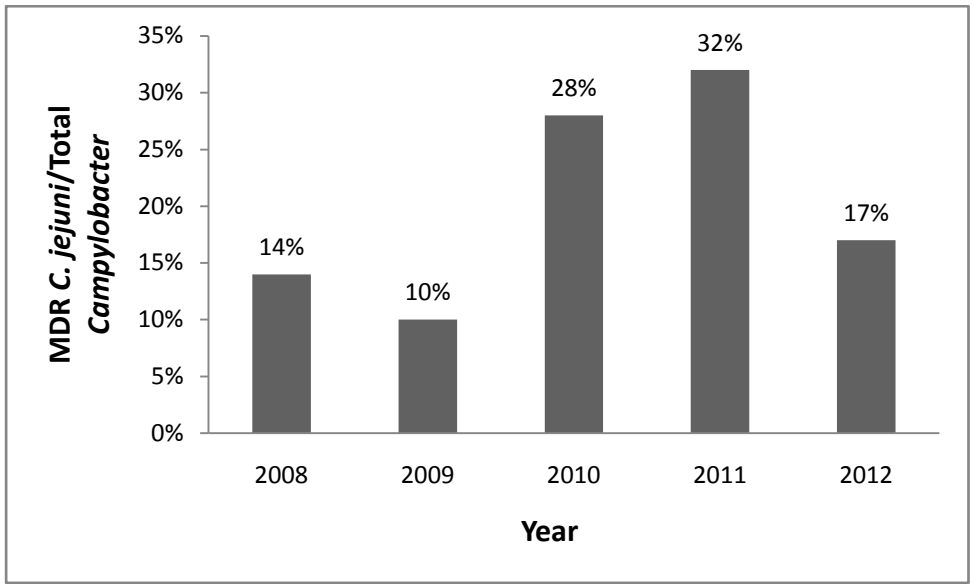


Figure 2.1. The percentages of MDR *C. jejuni* in total *Campylobacter* from four companies between 2008 and 2012.

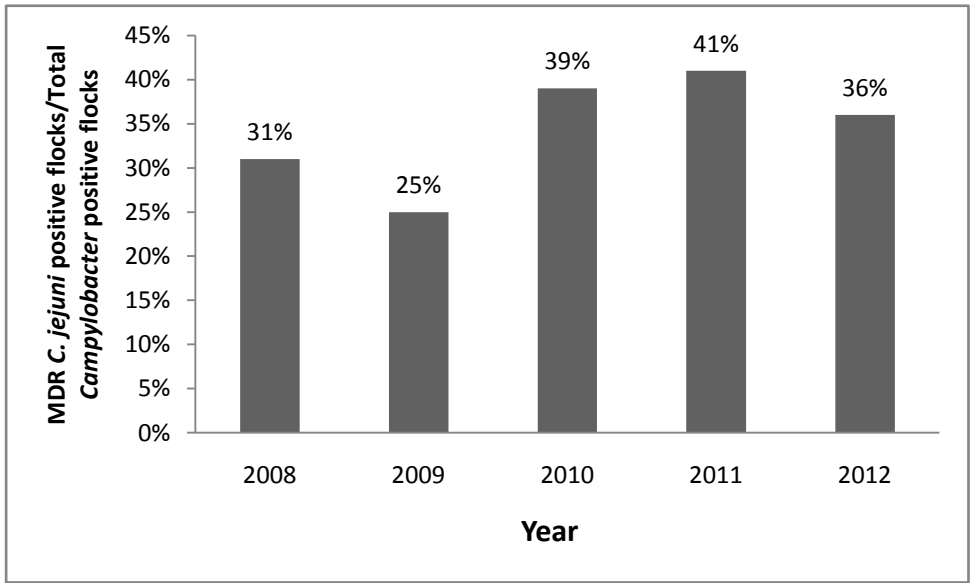


Figure 2.2. The percentages of MDR *C. jejuni*-positive flocks in total *Campylobacter*-positive flocks from four companies between 2008 and 2012.

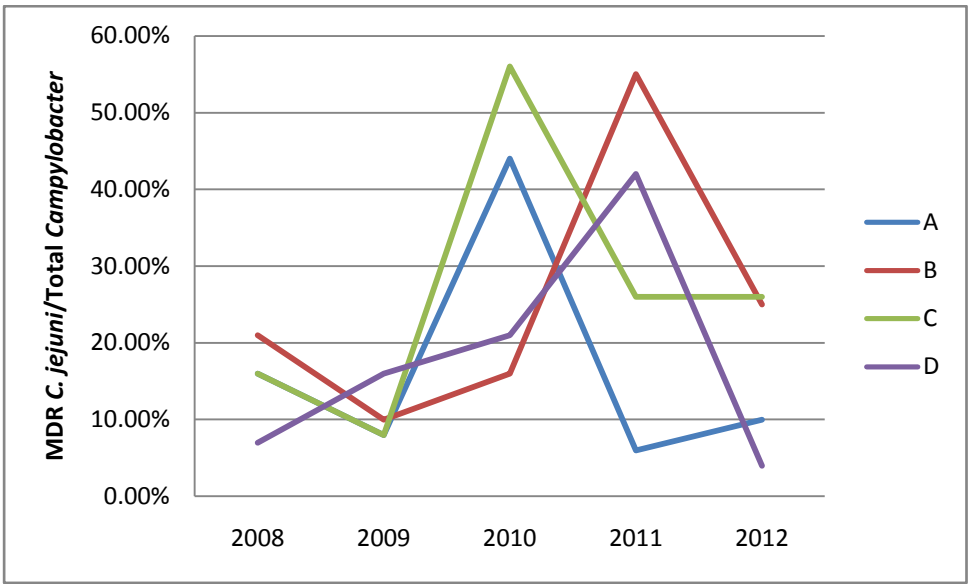


Figure 2.3. The percentages of MDR *C. jejuni* in total *Campylobacter* among four different companies between 2008 and 2012.

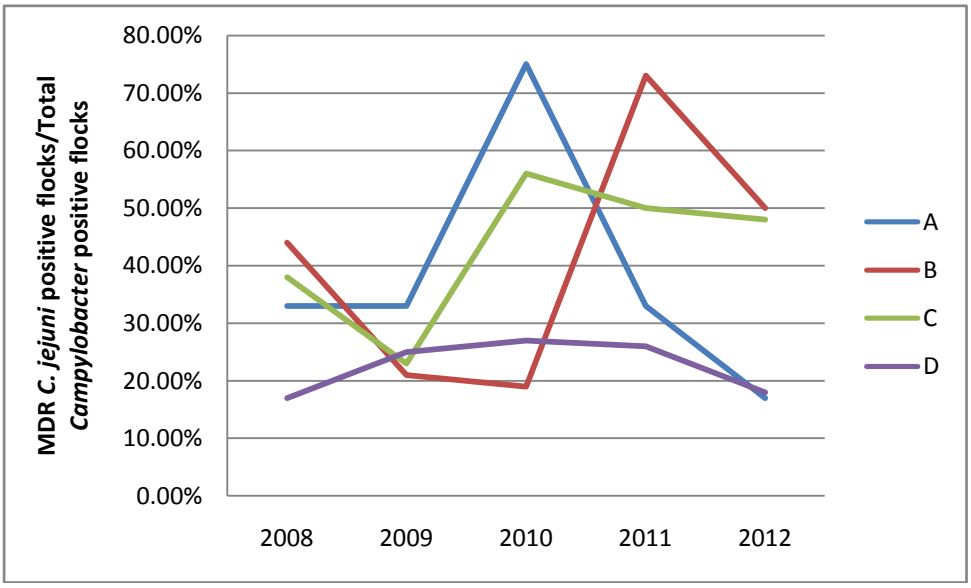


Figure 2.4. The percentages of MDR *C. jejuni*-positive flocks in total *Campylobacter*-positive flocks among four different companies between 2008 and 2012.

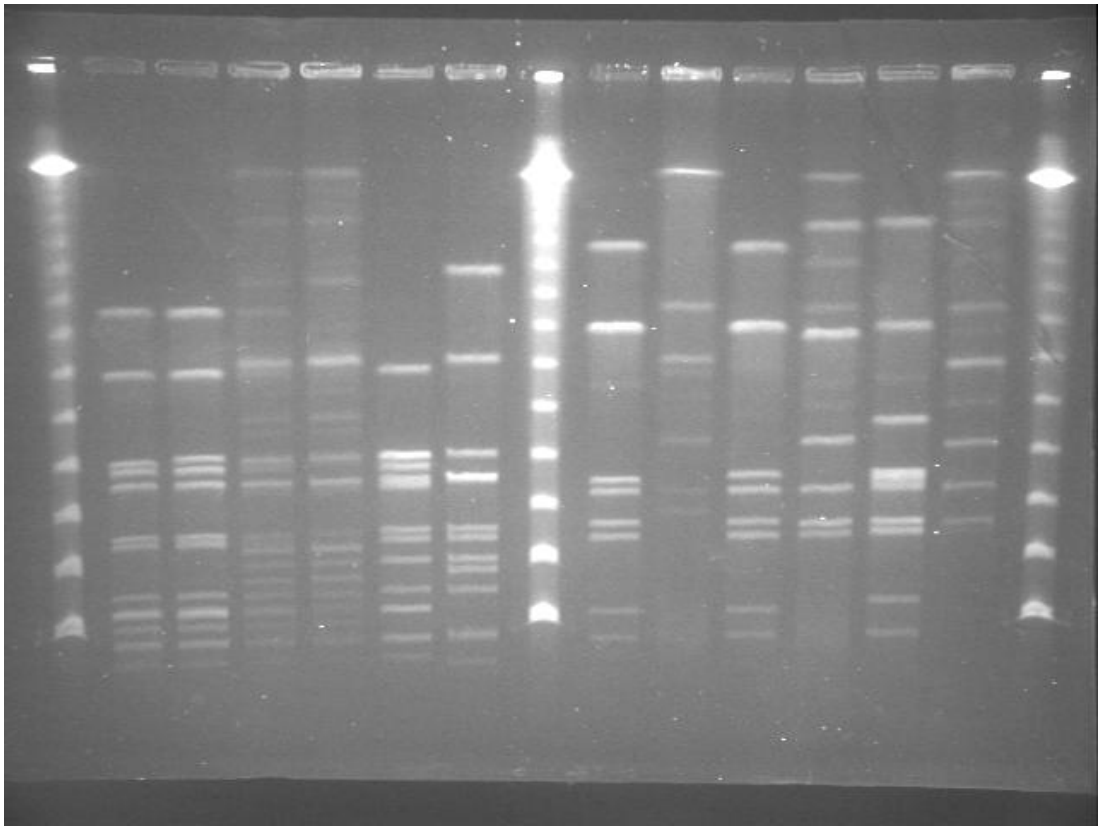
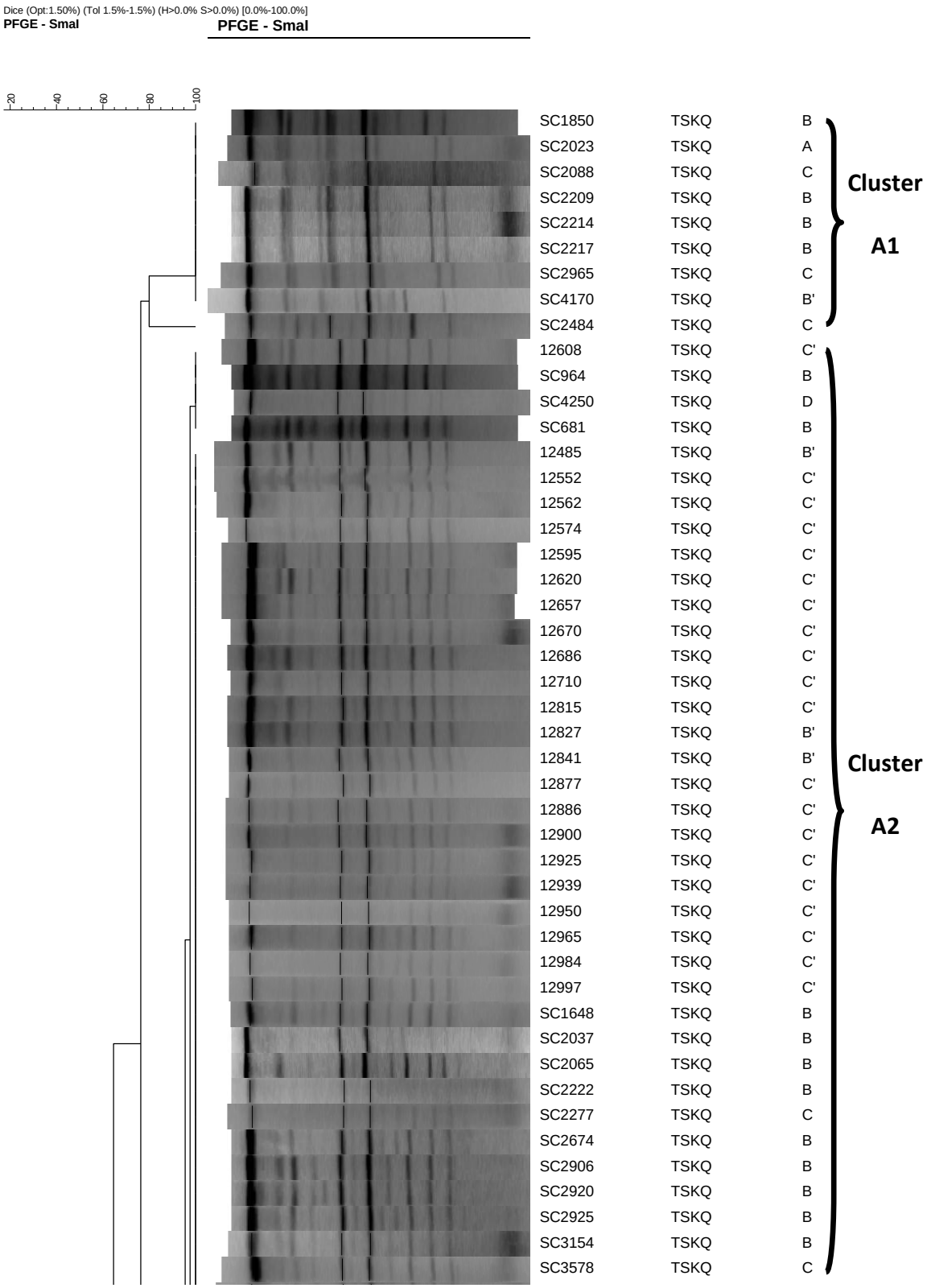
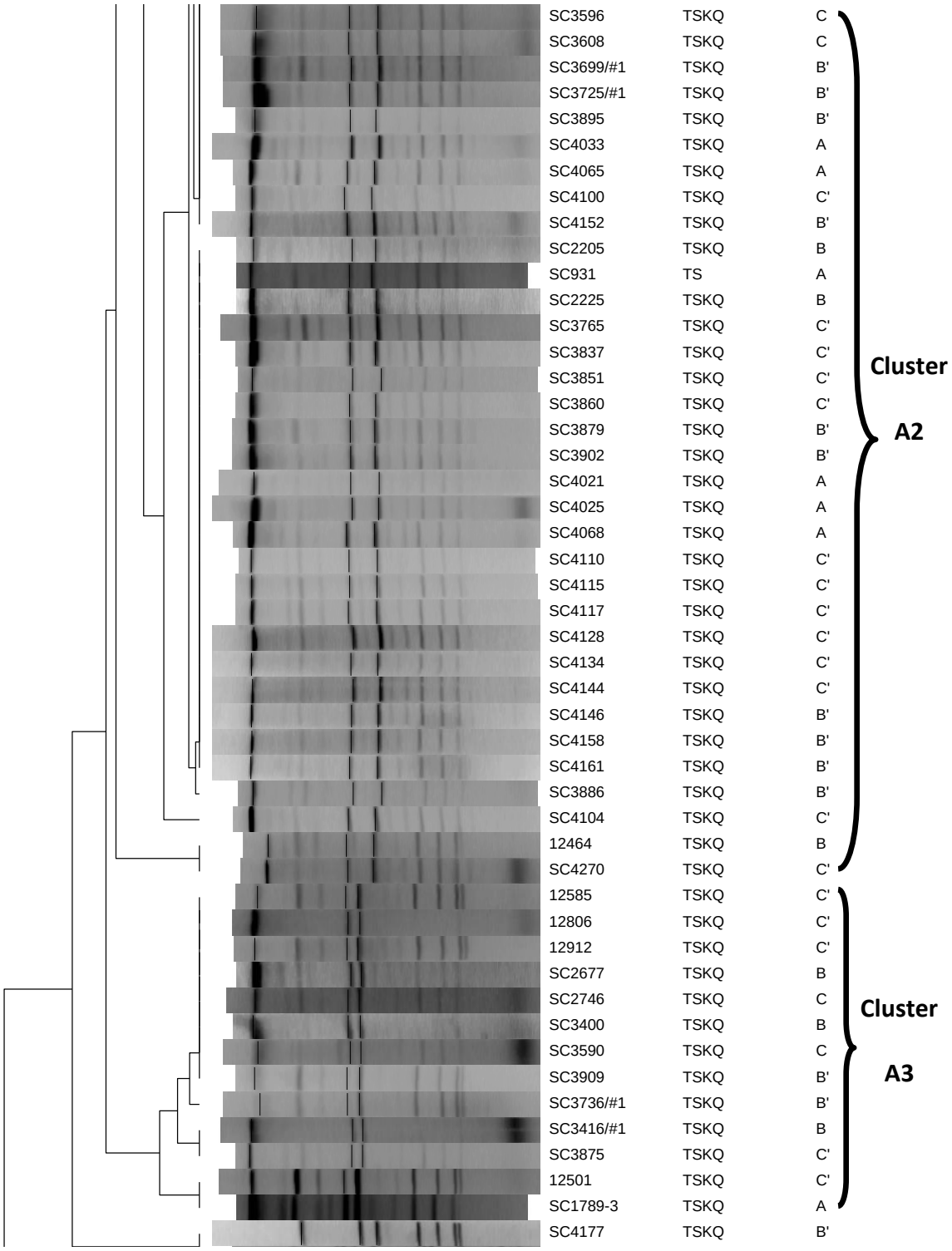
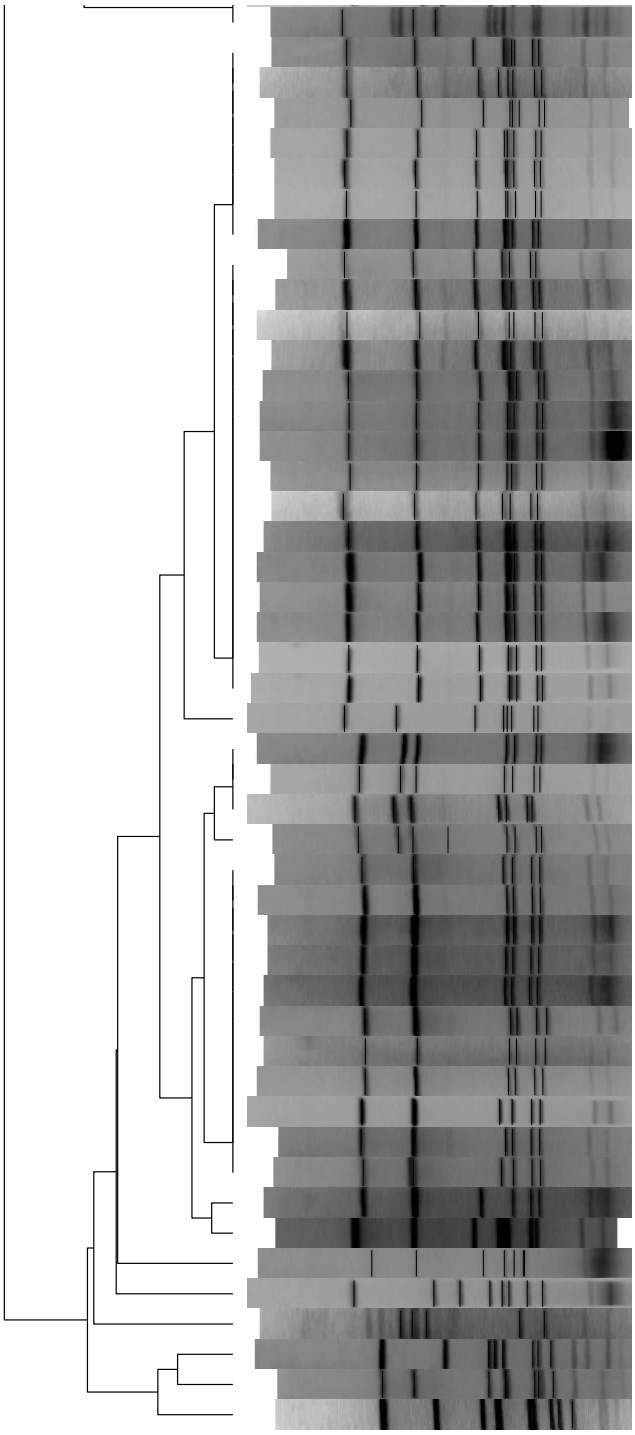


Figure 2.5. Representative pulsed-field gel electrophoresis of genomic DNA of *C. jejuni* digested by restriction enzyme SmaI. Lane 1, 8 and 15 are the Lambda ladder pulsed-field gel electrophoresis marker; lane 2-7 and 9-14 are *C. jejuni* isolates (lane 2 - SC4343, lane 3 - SC4357, lane 4 - SC4364, lane 5 – SC4366, lane 6 – SC4194, lane 7 – SC4213, lane 9 – 4218, lane 10 – SC4250, lane 11 – 12474, lane 12 – 12585, lane 13 – 12580 and lane 14 – 12574).

Figure 2.6. Dendrogram generated by PFGE (SmaI) profiles for 134 *C. jejuni* isolates investigated in this study. Cluster analysis was performed by BioNumeric (version 4.6, Applied Maths, Saint-Marten-Latem, Belgium).


Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]
PFGE - SmaI
PFGE - SmaI
20
40
60
80
100
SC1850 TSKQ B
SC2023 TSKQ A
SC2088 TSKQ C
SC2209 TSKQ B
SC2214 TSKQ B
SC2217 TSKQ B
SC2965 TSKQ C
SC4170 TSKQ B'
SC2484 TSKQ C
12608 TSKQ C'
SC964 TSKQ B
SC4250 TSKQ D
SC681 TSKQ B
12485 TSKQ B'
12552 TSKQ C'
12562 TSKQ C'
12574 TSKQ C'
12595 TSKQ C'
12620 TSKQ C'
12657 TSKQ C'
12670 TSKQ C'
12686 TSKQ C'
12710 TSKQ C'
12815 TSKQ C'
12827 TSKQ B'
12841 TSKQ B'
12877 TSKQ C'
12886 TSKQ C'
12900 TSKQ C'
12925 TSKQ C'
12939 TSKQ C'
12950 TSKQ C'
12965 TSKQ C'
12984 TSKQ C'
12997 TSKQ C'
SC1648 TSKQ B
SC2037 TSKQ B
SC2065 TSKQ B
SC2222 TSKQ B
SC2277 TSKQ C
SC2674 TSKQ B
SC2906 TSKQ B
SC2920 TSKQ B
SC2925 TSKQ B
SC3154 TSKQ B
SC3578 TSKQ C
Cluster A1
Cluster A2


SC3596 TSKQ C
SC3608 TSKQ C
SC3699/#1 TSKQ B'
SC3725/#1 TSKQ B'
SC3895 TSKQ B'
SC4033 TSKQ A
SC4065 TSKQ A
SC4100 TSKQ C'
SC4152 TSKQ B'
SC2205 TSKQ B
SC931 TS A
SC2225 TSKQ B
SC3765 TSKQ C'
SC3837 TSKQ C'
SC3851 TSKQ C'
SC3860 TSKQ C'
SC3879 TSKQ B'
SC3902 TSKQ B'
SC4021 TSKQ A
SC4025 TSKQ A
SC4068 TSKQ A
SC4110 TSKQ C'
SC4115 TSKQ C'
SC4117 TSKQ C'
SC4128 TSKQ C'
SC4134 TSKQ C'
SC4144 TSKQ C'
SC4146 TSKQ B'
SC4158 TSKQ B'
SC4161 TSKQ B'
SC3886 TSKQ B'
SC4104 TSKQ C'
12464 TSKQ B
SC4270 TSKQ C'
12585 TSKQ C'
12806 TSKQ C'
12912 TSKQ C'
SC2677 TSKQ B
SC2746 TSKQ C
SC3400 TSKQ B
SC3590 TSKQ C
SC3909 TSKQ B'
SC3736/#1 TSKQ B'
SC3416/#1 TSKQ B
SC3875 TSKQ C'
12501 TSKQ C'
SC1789-3 TSKQ A
SC4177 TSKQ B'
Cluster A2
Cluster A3



SC4356	TQ	A
12580	TK	C'
SC2272	T	C
SC3841	T	C'
SC3844	TK	C'
SC3873	T	C'
SC3881	T	B'
SC4221	T	D
12470	TSKQ	B'
SC2226	TK	B
SC2276	T	C
SC2934	T	B
SC2950	T	C
SC2982	TK	C
SC2988	T	C
SC3176	T	B
SC3404	T	B
SC3442/#1	T	C
SC3558/#1	T	B
SC3712/#1	T	B'
SC3764	T	C'
SC4019	T	A
SC4030	T	A
SC4042	T	A
SC3543/#1	Q	B
SC3843	TQ	C'
SC4150	TQ	B'
12941	TQ	C'
12474	TK	B'
12492	TK	C'
SC2715	TK	C
SC2727	TK	C
SC2745	-	C
SC2971	TK	C
SC3449	TK	C
SC3745	T	C'
SC4036	T	A
SC4218	TK	D
SC4307	T	B'
SC2756	TK	C
SC778	TK	C
SC3435-1/#1	-	C
SC4040	TS	A
SC2264	TK	C
12497	TSKQ	C'
SC4297	TK	B'
SC2045	T	B

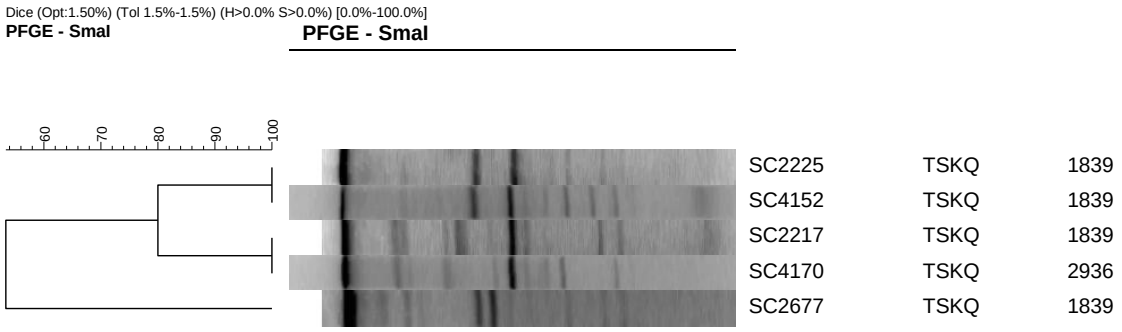


Figure 2.7. Dendrogram generated by PFGE (SmaI) profiles for 5 isolates typed by MLST.