ABSTRACT

AHMAD, HARUN. Antimicrobial Susceptibility Profiles and Clonal Population of Multidrug Resistant *Campylobacter coli* Isolates from Commercially Grown Turkeys. (Under the direction of Sophia Kathariou).

Campylobacter is one of the leading foodborne pathogens causing human gastroenteritis in the United States and other developed countries. *Campylobacter coli* currently contributes up to 15% of the total campylobacteriosis infections reported in the United States. *C. coli* commonly colonizes avian species, in agreement with many studies that suggested poultry as the main source of *campylobacter* infection to human. We focused our study on multidrug resistant (MDR) *C. coli* isolates from conventionally grown turkeys produced in this region, mostly between 2005 and 2009. A total of 152 MDR *C. coli* isolates were analyzed for antimicrobial minimum inhibitory concentrations (MIC) using the agar dilution method. The clonal population structure was determined using three genotyping techniques; pulsed-field gel electrophoresis (PFGE), *fla* typing, and multilocus sequence typing (MLST). The results of susceptibility tests against seven antimicrobial agents; ampicilin, ciprofloxacin, erythromycin, kanamycin, nalidixic acid, streptomycin, and tetracycline at concentrations from 0.5 to 256 μg/ml, showed that the majority of the MDR isolates were not inhibited at the highest used concentrations of ampicilin (92.8%), erythromycin (99.3%), kanamycin (98.7%), and streptomycin (99.3%). In regard to tetracycline, only five isolates were not inhibited in media containing 256 μg/ml tetracycline and the rest were inhibited at concentrations between 4 to 128 μg/ml. The found the highest MIC for ciprofloxacin was at 64 μg/ml and for nalidixic acid was at 128 μg/ml. The observed correlation at levels of

resistance to ciprofloxacin and nalidixic was expected, as both of these agents are from the same antimicrobial class (fluoroquinolones). However, a correlation was also observed between levels of resistance to the fluoroquinolones and to tetracycline, and this needs further investigation. We also found that there were company (integrator) effects on the level of resistance of MDR *C. coli* against certain antimicrobials agents. Future studies are required to determine the mechanisms irresponsible for the differences in levels of resistance among isolates from different companies. Analysis of the population structure of the 152 MDR *C. coli* based on the PFGE and *fla* typing revealed 77 different genotypes and majority (n=48) of them were unique genotypes. Five STs were identified in this study with four of them, the ST889, ST1101, ST1126, and ST1149 were turkey-specific and ST5131) is newly identified. We observed the presence of *CJE1461* gene in 113/178 (63%) of the MDR *C. coli* and 3/9 (33.3%) of the non-MDR *C. coli* based on PCR using *hip* primers. The noticed the prevalence of *CJE1461* gene in MDR *C. coli* isolates was increase from 27% in 2002 - 2004 to 81% in 2009. The *CJE1461* gene was previously identified as belonging to a "putative lysogenic bacteriophage that targeted tRNA-Met-1" in *C. jejuni* strain RM1221. The PFGE and *fla* analysis of the MDR *C. coli* isolates harboring this gene showed significantly different from those isolates without this gene. The findings from this study suggest that certain clonal group of MDR C. coli were prevalence in turkey produced in this region, and these groups continue harbor resistance to multiple antimicrobial agents. The prevalence of MDR *C. coli* strains in turkey production has the potential to give impact on risk the consumers. Further studies are required to closely monitor the changes in clonal population

of MDR isolates and determine the source of the CJE1461 gene and elucidate the reasons for its increasing prevalence in MDR *C. coli* from turkey flocks in this region.

Antimicrobial Susceptibility Profiles and Clonal Population of Multidrug Resistant *Campylobacter coli* Isolates from Commercially Grown Turkeys

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DEDICATION

To my lovely wife Nizar Abdullah @ Hanapi and all my four sweet children; Muhammad Hazim Taqiuddin, Amni Syafia, Adi Haikal, and Adam Muqris. My father Ahmad Hj. Dahlan and my mother Hafsah Umpang.

BIOGRAPHY

Harun Ahmad was born on October 5th, 1969 in Tawau, Sabah, Malaysia. He proudly attended the Sekolah Berasrama Penuh Sabah (S. B. P.S.) in Kota Kinabalu, Sabah from 1985 to 1987. He got his bachelor's degree in Microbiology at Universiti Kebangsaan Malaysia in 1992. He currently works with the Department of Chemistry under the Ministry of Science and Innovations (MOSTI) Malaysia since 1993. He has been awarded the study scholarship from the Department of Public Service Department of Malaysia to pursue his study in master degree in Department of Food, Bioprocessing and Nutrition Sciences at North Carolina State University under the mentorship of Dr. Sophia Kathariou. After obtaining his master, he will continue working with the Government of Malaysia.

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CHAPTER 1

Literature Review

1.1 *CAMPYLOBACTER*

1.1.1 Introduction

Campylobacter is derived from the Greek word "kampylos", which means curved. It was first isolated from a pregnant sheep by a veterinary pathologist, John McFadyean and his associate Stewart Stockman in Britain in 1906 (Skirrow, 2006). *Campylobacter* was initially classified with the genus *Vibrio*, mainly due to its curved or spiral shape that resembles the true *Vibrio* species, but later reclassified as *Campylobacter* spp. after the comprehensive taxonomic studies that used various biochemical and serological tests, and the G+C ratio in DNA by Veron and Chatelain (1973) (On, 2005). Progress on isolation and identification of *Campylobacter* species was slow because growth requires certain atmospheric conditions and specilized medium (Penner, 1988). However, rapid development and interest in this group has occurred after suitable media and techniques became available to isolate *Campylobacter* from clinical samples (Skirrow, 1977). Currently there are at least 16 species and six subspecies identified based on the 16S rRNA gene sequence comparisons (On, 2005). *C. coli* was closely related to *C. jejuni*, *C. lari*, and *C. upsaliensis*, *C. helveticus* (On, 2005)

1.1.2 General characteristics

Campylobacter is a non-spore forming, gram negative bacterium. It appears as curved, spiral or S-shaped cells with size from 0.2 to 0.8 μm wide and 0.5 to 5 μm long (Penner, 1988; Debruyne *et al*, 2008). Optimum growth for most species, including *C. jejuni* and *C. coli*, is under microaerobic conditions, but there are also species that grow optimally under anaerobic or aerobic conditions (Penner, 1988). Most *Campylobacter* spp. grow at

temperatures between 30°C and 37°C, but isolation of some thermophilic species is optimal at 42°C to 43°C (Penner, 1988; Debruyne *et al.*, 2008). *Campylobacter* is a motile microorganism with either one or two flagella, each located at the ends of the cell.

C. coli is closely related to *C. jejuni* and these two species were previously known as the "*C. jejuni-C. coli*" group (Skirrow, 1977). They both are catalase and nitrate positive, do not produce hydrogen sulfide (H_2S) in triple sugar iron (TSI) agar, indoxyl acetate positive, and prefer to grow at 42°C to 43°C (Penner, 1988). *C. coli* have close genetic properties with *C. jejuni* with which DNA-DNA hybridization values of 21-63% have been observed (On, 2005). According to a report by Penner (1988), in 1980, Harvey discovered that the hippuricase test could be used to differentiate *C. jejuni* from *C. coli*. He also found that *C. jejuni* is the only *Campylobacter* species that produce the enzyme hippuricase to hydrolyze hippurate to benzoic acid and glycine.

1.1.3 Infection and pathogenesis

Campylobacter is a zoonotic pathogen and can be transmitted to humans primarily via contaminated food, untreated drinking water, or raw milk (Adak *et al.*, 1995). Symptoms of campylobacteriosis include diarrhea, cramps, and fever, and nearly half of the patients have bloody diarrhea (Skirrow and Blaser, 2000; Friedman *et al.*, 2004). The most serious systemic illnesses caused by *C. jejuni* are reactive arthritis and the Guillain-Barré Syndrome (GBS), which is an acute immune system-mediated demyelinating disease of the peripheral nervous system (Blaser, 1997; Nachamkin et al., 1998). *Campylobacter* infection is mostly a self-limiting diarrheal illness, however in severe cases of campylobacteriosis such as when patients have recurrent or systemic *Campylobacter* infections, antimicrobial treatment will

have to be administered. The drugs of choice for severe *Campylobacter* infection are ciprofloxacin (class of fluoroquinolones) and macrolides such as erythromycin (Nachamkin *et al.*, 1998; Skirrow and Blaser, 2000).

1.1.4 Epidemiology

Campylobacter has been recognized as a leading bacterial cause of gastroenteritis in the United States and other industrialized countries (Friedman *et al.*, 2004; Mead *et al.*, 1999). A study in the United States estimated that ca. 2.5 million cases of *Campylobacter* infection occur each year and 80% of these are related to foodborne transmission (Mead *et al.*, 1999). However, due to under reporting the exact numbers could be five to 10 times higher than the reported figures (Lee and Newell, 2006).

A dose as low as 500-800 colony forming units of organisms is sufficient to produce illness in human (Robinson, 1981; Black *et al*., 1988; Medema et al., 1996). In foodborne infection by *Campylobacter*, between 50 and 70% are being transmitted from poultry and poultry products (Harris *et al*., 1986; Allos, 2001; Keener et al, 2004).

Recent US Centers for Disease Control and Prevention (CDC) Foodborne Disease Surveillance Network (FoodNet) data suggest that *Campylobacter* is the second leading bacterial cause of human gastroenteritis infection in the United States, and it accounts for ca. 13 infections per 100,000 persons (CDC, 2009a). Of the confirmed clinical isolates from *Campylobacter* infections from 1997 to 2001 95% were *C. jejuni*, 4% were *C. coli*, and the rest were *C. upsaliensis*, *C. lari*, *C. fetus*, and *C. mucosalis* (Friedman *et al.*, 2004).

Most of the *Campylobacter* infections are sporadic and the potential sources of human infection are contaminated meat, poultry, milk, water and contact with animals (Neimann *et*

al., 2003). According to the CDC (2009a), children below age 4 years old and persons over 50 years old had the highest risk for infection.

In animals, *Campylobacter* is a commensal bacterium that can easily be found in cattle, sheep, swine and mostly in avian species (Skirrow, 1977). Several studies have demonstrated that *Campylobacter* could not be isolated from day-old birds (Lee *et al*., 2005; Smith *et al.*, 2004), however it becomes a common bacterium in the avian gut and can be detected as early as 2 weeks post-hatch (Bull, 2006). In a positive flock, *Campylobacter* could colonize all birds in just few days (Newell and Fearnley, 2003; Lee *et al*., 2005). The prevalence of *Campylobacter coli* in isolates from commercially grown turkey flocks was between 80 and 90% of the total Campylobacter-positive samples (Smith *et al.*, 2004). Some studies have shown that extensive colonization by strains with multidrug resistance are frequently observed in conventional poultry production and could represent up to 81% of the total isolates of *Campylobacter* strain (Lee *et al*., 2005; Luangtongkum *et al*., 2006).

Campylobacter infection is normally associated with drinking unpasteurized milk or untreated water, and with consumption of meat and poultry products (Studahl and Andersson, 2000). The major risk for foodborne infection was found to be from the handling and consumption of poultry products (Altekruse and Tollefson, 2003).

Since *Campylobacter* is quite prevalent in poultry and meat products, cross contamination normally occurs from the contaminated meat to other foods during preparation in the home kitchen. Foods prepared in restaurants have also been associated in nearly half of the total sporadic *Campylobacter* infections in the United States (Friedman *et al.*, 2004). To prevent cross contamination with bacteria from poultry and meat products in the kitchen it is

recommended that good hygiene practices and safe-food handling procedures are followed at all times (CDC, 2009a; Anne *et al.*, 2008).

1.2 GENOTYPING TECHNIQUES

There are two serotyping schemes widely used for *Campylobacter*, the Penner scheme and the Lior scheme. The Penner scheme uses heat-stable (HS) antigens while the Lior scheme uses the heat-labile (HL) antigens, such as flagellin. Both techniques have limited discriminatory power and are time-consuming and technically demanding; for these reasons they are not suitable for large-scale screening and epidemiologic studies. Molecular subtyping tools have been used since the 1980s to determine the genetic polymorphisms in *Campylobacter* spp. On *et al.* (2008) suggested that with the latest developments in molecular technology, deoxyribonucleic acid (DNA) sequence-based subtyping would be more discriminatory and useful in subtyping various *Campylobacter* species. There are many types of methods for bacterial subtyping in surveillance and epidemiologic studies. Restriction fragment length polymorphism (RFLP) (including PCR fragment RFLP such as *fla* typing), polymerase chain reaction (PCR), Ribotyping, pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), DNA sequencing, multilocus sequence typing (MLST), and DNA arrays are some of the methods currently available.

1.2.1 Pulsed-Field Gel Electrophoresis (PFGE)

PFGE is generally considered as the most discriminatory typing method commonly available for genotyping or genetic fingerprinting of bacteria (On *et al*., 2008). PFGE is

currently being used by PulseNet, the national network of public health and food regulatory agency laboratories coordinated by the CDC, and all of their participating laboratories to perform standardized molecular subtyping or fingerprinting for foodborne disease bacteria (CDC, 2009). This technique was developed in 1984 by Schwartz and Cantor (1984) at Columbia University and involves an alternating voltage gradient that improved the resolution of large DNA molecules. The whole genome from cells embedded in agarose was cut using restriction enzymes to produce a few fragments, typically between 4 and 10.

PFGE is quite similar to regular gel electrophoresis except that instead of running the voltage in one direction, the voltage in PFGE is switched in three directions periodically. This facilitates the movement of DNA fragments larger than 15-20kb.

According to Wassenaar and Newell (2000), there could be differences in the conditions for PFGE and the enzymes used among laboratories. They also reported that there is a number of restriction enzymes that could be used for this analysis, including *Sma*I, *Sal*I, *Kpn*I, *Apa*I, and *Bss*HII. They also reported that comparisons among different laboratories are difficult to make because of the differences in PFGE conditions and restriction enzymes used.

PFGE was used in many studies to genotype *Campylobacter* spp isolated from turkey flocks, human isolates, and poultry (e.g. De Boer *et al*., 2000; Michaud *et al*., 2001; D'lima *et al*., 2007). PFGE analysis requires many steps and is time consuming and not all laboratories have the facilities and expertise to perform this analysis. PFGE is at present considered as the "gold standard" in molecular epidemiology studies.

1.2.2 Multilocus sequence typing (MLST)

In *Campylobacter* studies, MLST method was originally developed to type *C. jejuni* (Dingle *et al*., 2001). This technique characterizes isolates of *Campylobacter* species based on the sequence of internal fragments of seven house-keeping genes: *aspA*, *atpA*, *glnA*, *gltA*, *glyA*, *pgm*, and *tkt* (Dingle *et al*., 2001). Currently, this technique has been used to identify several host-associated alleles in *C. coli* isolated from food animals such as cattle, chicken, swine, and turkeys (Miller *et al.*, 2006).

Disadvantages of MLST include the cost and the fact that discriminatory power is lower that with PFGE. However, MLST has proved to be better than other molecular typing schemes because the sequence data are portable, inherently reproducible and are readily compared among laboratories, especially via the internet (Sheppard and Dallas, 2009).

1.2.3 *Fla* Typing

C. coli has two flagellin genes, *flaA* and *flaB*, each about 1.7 kb and separated from each other by approximately 170 bp. These genes are responsible for the development of flagella that can be found on one or both ends of the *Campylobacter* cells. *Campylobacter* flagellin genes have highly conserved and variable regions, especially at the 5' and 3' ends, that make them suitable for restriction fragment length polymorphism (*fla*-RFLP) analysis (Meinersmann *et al*., 1997). On *et al.* (2008) discovered that the primers described by Nachamkin *et al* (1993) that amplify 1,728 bp of the flagellin A gene are optimal for *fla* typing.

To identify variability in *fla* sequence, restriction enzymes *Alu*I, *Dde*I, *Eco*RI, *Hin*fI and *Pst*I are currently used alone or in various combinations. While *Hin*fI is not sufficiently

discriminatory and *Alu*I generates bands that are too small, *Dde*I appears to produce the best discriminatory power and is mostly preferred because it is easy to perform, less expensive, and could be used in high throughput analysis (On *et al*., 2008). The discriminatory power could be further improved by combining *Dde*I with *Hin*fI (Wassenaar and Newell, 2000). Even though there are reports that show that *flaA*-RFLP was less discriminatory than MLST this method proved to be a useful technique for epidemiological studies and strain discrimination in an outbreak analysis (On *et al.*, 2008; Clark *et al*., 2005; Wassenaar and Newell, 2000).

1.2.4 Ribotyping

Ribotyping is a sensitive and precise method that can be used to identify and classify bacteria. Analysis of 16S regions using restriction enzymes generates rRNA gene patterns that can be used to distinguish organisms within the same species. In *Campylobacter*, the enzyme *Pst*I is normally used to cut the 16S rRNA gene region producing between three and six bands (On *et al.*, 2008). Fitzgerald *et al.* (1996) have used *Pst*I and *Hae*III to genotype *C. jejuni* isolated of human and animal origin. They found that digestion using *Pst*I produced bands between 5.9 and 30 kbp, and *Hae*III produced bands between 1.0 and 20 kpb.

Campylobacter only has three copies of ribosomal RNA genes (On *et al.*, 2008). Some studies have demonstrated that ribotyping is less discriminatory compared to *Sma*I PFGE or MLST (Ge *et al.*, 2006; O'Rilley *et al.*, 2006). Thus, there is limited use of ribotyping in *Campylobacter* typing due to the low level of diversity and the high cost of automated ribotyping compared to *Sma*I PFGE or *flaA*-RFLP typing (On *et al.*, 2008).

Manfreda *et al.* (2003) suggested that ribotyping could be used with other fingerprinting methods to characterize *Campylobacter* isolates.

1.3. MULTIDRUG RESISTANCE IN *CAMPYLOBACTER COLI*

1.3.1 General mechanisms

It is being speculated that there were no antibiotic-resistant microbes isolated from humans or animals before the introduction and use of antibiotics. In most cases, soon after the antibiotic is used for clinical practice, pathogenic bacteria start to develop resistance (Mazel and Davies, 1999). Bacteria could develop resistance to antibiotics either due to mutation or by getting the resistance genes from exogenous sources (Davis, 1994). Antibiotic resistance genes among bacteria are mostly being transferred horizontally (Mazel and Davies, 1999).

1.3.2 Fluoroquinolone resistance

Fluoroquinolones were introduced in the United States in 1986 to treat human infections (Altekruse and Tollefson, 2003; Nelson *et al*., 2007). In 1995 and 1996, the United States Food and Drug Administration (FDA) approved 2 types of fluoroquinolones - sarafloxacin and enroloxacin, to treat avian colibacillosis in poultry flocks (Altekruse and Tollefson, 2003, Nelson *et al*., 2007). Fluoroquinolones are chemotherapeutic bactericidal drugs that inhibit the bacterial enzyme DNA-gyrase (topoisomers), which is important for the supercoiling of chromosomal DNA. Without DNA-gryase, the DNA replication is disrupted and this prevents the bacteria from multiplying.

Hooper *et al.* (1987) suggested that bacteria could become resistant to fluoroquinolones when the fluoroquinolones-binding site in the DNA-gyrase subunit A was altered due to a spontaneous point mutation. Other studies have shown that resistance may also be due to the failure of the drugs to enter the cell (Taylor *et al.*, 1988). Several studies have concluded that fluoroquinolone resistance in *Campylobacter* was mostly due to a spontaneous single point mutation in the gyrase gene *gyrA* that caused an amino acid substitution. These studies also suggested that alteration in the fluoroquinolone-binding site prevented the antimicrobial from inhibiting DNA-gyrase (Hooper *et al.*, 1987; Taylor *et al.*, 1988).

Friedman *et al.* (2000) reported that before the introduction of fluoroquinolones for human use in the United States in 1986, there were no fluoroquinolone-resistant *Campylobacter* strains among human isolates. The resistant strains only started to appear one year after the introduction for human use and the rate increased significantly when the FDA approved fluoroquinolone use in the poultry industry (Altekruse and Tollefson, 2003). Nelson *et al.* (2007) observed that infection by fluoroquinolone-resistant *Campylobacter* strains could increase the duration of symptoms and the likelihood of hospitalization. The FDA has determined that the use of fluoroquinolones in poultry farming has partially contributed to the compromise of the clinical utility of fluoroquinolones in humans.

The data from various studies suggested that the increase in human infections with fluoroquinolone-resistant *Campylobacter* was related to poultry consumption (Nelson *et al.*, 2007; Smith and Fratamico, 2010). In reaction to the problem of fluoroquinolone-resistant *Campylobacter* strains as a cause of human infections in the United States, in 2005 the FDA

prohibited the use of fluoroquinolones in the poultry industry. According to National Antimicrobial Resistance Monitoring System (NARMS), *Campylobacter* isolates able to grow in medium containing ciprofloxacin > 4 μg/ml were considered to be resistant (CDC, 2009).

1.3.3 Macrolide (including erythromycin) Resistance

Erythromycin is in the class of macrolide antibiotics which when used against bacteria interfere with the production of proteins. Erythromycin prevents protein synthesis by inhibiting the translocation step that is needed for the elongation of the peptide chain (Gibreel and Taylor, 2006).

A study by Gibreel and Taylor (2006) suggested that macrolides attach to 23s rRNA at nucleotide A2058 and this obstructs the entrance to the tunnel in the large ribosome subunit, thus preventing the translocation step that in needed for the elongation of the peptide chain. Resistance towards erythromycin has been reported to be associated with various mechanisms including target modification, enzymatic inactivation of the macrolides, or enhanced efflux of the drugs (Gibreel and Taylor, 2006). Several reports have shown that the methylation by rRNA methylase at position 22058 of the 23s rRNA could prevent the attachment of erythromycin to its target (Nakajima, 1999; Poehlsgaard and Douthwaite, 2005). A specific point mutation in the 23S rRNA of *Campylobacter* had also been associated with the macrolide resistance (Vacher *et al*., 2003). Macrolide resistance due to the mutation in the 50S ribosomal subunit proteins L4 and L22 has also been reported (Gibreel and Taylor, 2006). Payot *et al.* (2004) discovered that there are two groups of erythromycin-resistant *Campylobacter* isolates based on the mutation in the 23S rRNA gene.

The low level group is those without mutations in the 23S rRNA target gene, while the high level group always has a point mutation in the 23S rRNA gene.

Kim *et al.* (2006) conducted a study on erythromycin resistance *C. coli* strains isolated from commercially grown turkeys and swine. They discovered that DNA transformation played a major role in the acquisition of high-level resistance to erythromycin in animal-derived *C. coli*. They also discovered that the frequency of DNA transformation of strains isolated from turkeys was significantly higher than of strains isolated from swine.

The role of the Cme-ABC efflux pump in both intrinsic and acquired resistance to erythromycin, in both *C. jejuni* and *C. coli* has also been reported (Payot *et al*., 2004; Gibreel *et. al*., 2005 Mamelli *et al*., 2005). The NARMS program has considered *Campylobacter* strains able to grow in medium containing > 32 μg/ml of erythromycin to be resistant (CDC, 2009)

1.3.4 Tetracycline Resistance

Tetracycline is a broad-spectrum antibiotic used to treat animals against infection from both aerobic and anaerobic gram-negative and gram-positive bacteria. This water soluble antibiotic has also been used in food animals for therapeutic treatment and as growth promoter, especially on commercial farms (Chopra and Roberts, 2001). Tetracycline inhibits bacterial growth by preventing protein synthesis. Once inside the bacterium, tetracycline binds reversibly to the ribosome acceptor (A) site, thus preventing the attachment of aminoacyl-tRNA (aa-tRNA) (Chopra and Roberts, 2001). Without aminoacyl-tRNA supplying amino acid to the ribosome, the elongation process in protein synthesis will stop (Taylor and Tracz, 2005).

In general, there are four major categories of bacterial resistance to tetracycline; ribosome protection mechanism, efflux pumps, modification of the antibiotics, and mutation of the ribosome (Connell *et al*., 2003a; Zhang and Plummer, 2008). However, for *Campylobacter* most studies had focused on the ribosome protection mechanism and the efflux system (Zhang and Plummer, 2008). As reported by Taylor and Courvalin (1988) resistance is due to the production of Tet(O) protein that binds to the ribosome, thus inhibiting the function of tetracycline inside the bacterial cell. Based on structural analysis, association of tetracycline with the ribosome changes the structure of the 16 S rRNA (Noah *et al*., 1999; Chopra and Roberts, 2001). Tet(O) also interacts with and binds to the post-translocation ribosome allowing protein synthesis in the presence of tetracycline (Connell *et al.*, 2003).

In *C. jejuni tet*(O) is mainly encoded in plasmids and transferable via conjugation (Taylor *et al*., 1987; Chopra and Roberts, 2001; Gibreel *et al.*, 2004). Some *Campylobacter* strains encode *tet*(O) gene in their chromosome (Zhang and Plummer, 2008). Resistance to tetracycline is due to genetic acquisition of *tet*(O) genes (Chopra and Roberts, 2001).

Extensive use for therapeutic applications and growth promotion in farm animals might have contributed to the increased bacterial resistance to tetracycline, especially in *Campylobacter* (Chopra and Roberts, 2001; Gibreel *et al*., 2004). In commercial farms, antibiotics are normally added directly to feed or water for therapeutic treatment to treat disease as well as for growth promotion (Chopra and Roberts, 2001). The combination of the CmeABC efflux system and the Tet(O) protein has resulted in high-level tetracycline resistance in *Campylobacter* (Taylor and Tracz, 2005; Gibreel *et al*., 2007).

1.3. 5 Aminoglycoside (Kanamycin and Streptomycin) Resistance

Aminoglycosides are a group of narrow-spectrum antibiotics that are particularly active against a wide-range of aerobic Gram-negative bacteria, staphylococci and certain mycobacteria. Included in this group are kanamycin and streptomycin. These antibiotics have rapid bactericidal activity and are usually administered parenterally by either intravenous or intramuscular routes.

Aminoglycosides bind particularly to the 30S ribosomal subunit and interfere with protein synthesis (Jana and Deb, 2006). These antibiotics disrupt the elongation process and interfere with the normal proofreading activity of the ribosome. Proteins are not formed or are translated inappropriately (Zhang and Plummer, 2008).

There are four aminoglycoside resistance mechanisms in bacteria: 1.Reduction of the intracellular concentration of antibiotic inside the bacteria cell either by general efflux pump mechanism or by the reduced permeability of the antibiotic through the membrane (Jana and Deb, 2006); 2. Modification of the molecular target, normally due to mutation at the binding site of the ribosomal RNA; 3, Methylation of the 16S rRNA molecule at specific positions crucial for the attachment of the drug (Maravic, 2004; Magnet and Blanchard, 2005); and 4, Enzymatic inactivation of the drug, which is the major cause for amyloglycoside resistance in *Campylobacter* species (Jana and Deb, 2006; Zhang and Plummer, 2008).

Aminoglycosides are susceptible to three major types of enzymes; aminoglycoside acetyltransferases (acetylation enzymes), aminoglycoside nucleotidyltransferases that include adenylyltransferase (adenylating enzymes), and aminoglycoside phosphotransferases (phosphorylating enzymes) (Jana and Deb, 2006; Alfredson and Korolik, 2007). All these

enzymes produce 3'-O-aminoglycoside phosphotransferase (APH(3)) that covalently modify and inactivate these antibiotics (Jana and Deb, 2006; Alfredson and Korolik, 2007).

Aminoglycoside acetyltransferases and aminoglycoside phosphotransferases are both encoded on plasmids (Taylor and Courvalin, 1988; Jana and Deb, 2006; Ouellette *et al*., 1987). Several studies on *C. jejuni* and *C. coli* had concluded that the kanamycin-resistance phosphotransferase gene has been acquired from Gram-positive streptococci (Taylor and Courvalin, 1988; Tenover *et al*., 1989; Gibreel *et al*., 2004a). Aminoglycoside adenyltransferase is encoded in the bacterial chromosome and is believed to have originated from Gram-negative *Enterobacteriaceae* (Ouellette *et al*., 1987; Taylor and Courvalin, 1988).

Enzymatic modification to aminoglycoside drugs by APH(3) is the main contributor to the aminoglycoside resistance in *Campylobacter* spp. (Zhang and Plummer, 2008). Both kanamycin-resistance and tetracycline resistance genes were normally being transferred via conjugative plasmids (Taylor and Courvalin, 1988; Gibreel *et al*, 2004a). Based on the study of *C. coli* BM2509, streptomycin resistance is related to the production of 3"-9-Aminoglycoside nucleotidyltransferase (Taylor and Courvalin, 1988).

1.3.6 B-lactam Resistance (penicillin group)

Ampicilin is commonly used to treat bacterial infection such as from *Escherchia coli*, *Salmonella*, *Shigella*, Streptococci and certain strains of staphylococci. This antibiotic inhibits the formation of the cell wall, especially during the multiplying stage, thus preventing the bacteria from growing.

Aerestrup and Engberg (2001) reported that bacteria could develop resistance to B-lactams either by producing B-lactamase enzymes that hydrolyzed the antibiotic or by developing cell walls that could block the intake of this antibiotic into the cell. It has been suggested that resistance in *Campylobacter* strains appear to be chromosomally encoded (Taylor and Courvalin, 1988).

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CHAPTER 2

Antimicrobial Resistance Profiles and Genetic Diversity of Multidrug Resistance *Campylobacter coli* Isolates from Commercially Grown Turkeys

2.1 ABSTRACT

The widespread use of antimicrobial agents in both humans and animals can result in the emergence of antimicrobial resistance in pathogens. *Campylobacter* currently is one of the most common foodborne pathogens in the United States and the National Antimicrobial Resistance Monitoring System (NARMS) has monitored its antimicrobial susceptibility since 1997. In this study, we characterized 152 multidrug resistant *C. coli* isolated from commercially grown turkeys between 2006 and 2009 in terms of antibiotic resistance levels and strain genotype. The isolates were tested for minimum inhibitory concentration (MIC) to seven antimicrobials (ampicilin, erythromycin, kanamycin, streptomycin, nalidixic acid, ciprofloxacin, and tetracycline) by the agar dilution method. The majority of the isolates were highly resistant (>250 μg/ml) to ampicilin (92.8%), erythromycin (99.3%), kanamycin (98.7%) and streptomycin (99.3%). All isolates were inhibited at 128 μg/ml of nalidixic acid, or 64 μg/ml of ciprofloxacin. We found that there was linear correlation in resistance to ciprofloxacin and nalidixic acid ($p = 0.0221$), and to ciprofloxacin and tetracycline ($p < 0.0001$). The company effect that produced the birds (n=5) was a significant main effect for resistance to ciprofloxacin ($p = 0.002$) and nalidixic acid ($p < 0.001$). The genotype for our MDR *C. coli* isolates were determined by pulsed-field gel electrophoresis with *Sma*I, *fla*-typing, and multilocus sequence typing (MLST). A total of 77 different genotypes were identified based on the *fla* and PFGE typing. We found four turkey-specific sequence types (STs); ST889, ST1101, ST1126, ST1149 and the ST5131 was newly identified in this study, suggesting that turkeys produced in this region were consistently colonized by certain clonal groups of *C. coli*.

2.2 INTRODUCTION

Campylobacter has been recognized as a leading bacterial cause of gastroenteritis in the United States and other industrialized countries (Mead *et al.*, 1999). A study in the United States estimated that ca. 2.5 million cases of *Campylobacter* infection occurred each year and 80% of these are related to foodborne transmission (Mead *et al.*, 1999). Reports by the FoodNet have shown that in the United States, the number of infections by *Campylobacter* was 12.78 per 100,000 population, which was second (after *Salmonella*) (http://www.cdc.gov/foodnet/annual/2007/2007_annual_report_508.pdf).

Most *Campylobacter* infections are sporadic and the potential sources of human infection are contaminated meat, poultry, milk, water and direct transmission from animals (Neimann *et al.*, 2003). Symptoms associated with campylobacteriosis are diarrhea, cramps, and fever, and nearly half of the patients report bloody diarrhea (Friedman *et al.*, 2004; Skirrow and Blaser, 2000).

Campylobacter infection is mostly a self-limiting diarrheal illness, however antimicrobial treatment will have to be administered in severe cases of campylobacteriosis, such as with patients having recurrent or systemic *Campylobacter* infection. The drugs of choice for severe *Campylobacter* infection are ciprofloxacin (class of fluoroquinolones) and macrolides such as erythromycin (Nachamkin *et al.*, 1998; Skirrow and Blaser, 2000). *C. jejuni* is responsible for most (ca. 85%) human infections, with *C. coli* associated with most (ca. 15%) of the remainder (Friedman *et al.*, 2004).

Several studies have demonstrated that the high prevalence of *Campylobacter* in poultry products has contributed significantly to human infection (Ikram *et al.*, 1994; Jore *et*

al., 2010; Friedman *et al.*, 2004). Recent reports showed that only 1.6% of ground turkey samples in the United States were contaminated with *Campylobacter* compared to 49.9% of chicken breast samples (Zhao *et al*., 2010). However, isolates from turkey products were more likely to have resistance to many types of antimicrobial agents than isolates from chicken (Ge *et al*., 2003). *C. coli* isolates are more resistant than *C. jejuni* to most antimicrobial agents, except for tetracycline (Aaaestrup, 2005; Ge *et al*., 2003; Luangtongkum *et al*., 2006; Zhao *et al*., 2010).

Campylobacter commonly colonizes the avian gut and it can be detected in chickens as early as 2 weeks post-hatch (Bull *et al.*, 2006). Previous studies from our laboratory showed that multidrug resistant (MDR) *C. coli* frequently colonized turkey flocks in eastern North Carolina (D'lima *et al*, 2007). *Campylobacter* could not be isolated from the ceca of day-old birds but after day 14 the turkey flocks appeared to be colonized by *Campylobacter* (Lee *at al*., 2005). The prevalence of *Campylobacter coli* in isolates from turkey flocks was between 80 and 93% of the total Campylobacter-positive samples (Smith *et al.*, 2004; Wright *et al.*, 2008).

Antimicrobial agents are generally used in veterinary practice for therapy, prophylaxis, and growth promotion (McEwen and Fedorka-Cray, 2002; Aarestrup, 2005). The prevalence of antimicrobial resistance in *Campylobacter* has been reported in many studies. Resistance to ampicillin, deoxycycline, erythromycin, ciprofloxacin, nalidixic acid, tetracycline, and trimethoprim-sulfamethoxazole has been reported in *Campylobacter* isolates from poultry meat (Ge *et al*., 2003; Luber *et al*, 2003). Multidrug resistant *C. coli*

and *C. jejuni* resistant to more than six types of antimicrobials agents were frequently isolated from poultry (D'lima *et al*., 2007; Lee *et al*., 2005; Luangtongkum *et al*., 2006).

This research will identify the strains that currently colonize turkeys in commercial farms from four companies (integrators) in Eastern North Carolina and one company in South Carolina. This study will also look into the strain diversity of MDR *C. coli*, and the level of resistance of the isolates to seven antimicrobial agents; Ampicillin, ciprofloxacin, erythromycin, kanamycin, nalidixic acid, streptomycin, and tetracycline. We applied pulsed-field gel electrophoresis (PFGE), *flaA* typing and multilocus sequence typing (MLST) methods to determine the genetic diversity of isolates. Combinations of PFGE and *flaA* typing have been used successfully in several studies to determine the genetic profiles of *C. jejuni* and *C. coli* (D'lima *et al.*, 2007; Gu *et al.*, 2009; Fitzgerald *et al.*, 2001).

2.3 MATERIALS AND METHODS

***Campylobacter* strains and growth condition.** The MDR *C. coli* isolates used in this study was selected from our laboratory *Campylobacter* strain collection and are listed in Table 2.1. Isolates were defined as MDR based on breakpoints of ≥100 µg/ml for ampicilin, ≥10µg/ml for erythromycin, ≥20 µg/ml for kanamycin, ≥10 µg/ml for tetracycline, ≥15 µg/ml for streptomycin, ≥20 µg/ml for nalidixic acid, and ≥4 µg/ml for ciprofloxacin. *C. coli* strains had been isolated as described (Smith *et al.*, 2004; Wright *et al.*, 2008) from commercially grown turkeys in North Carolina and South Carolina between 2006 and 2009. Turkeys were from 110 farms operated by five different companies (integrators). Bacteria

were preserved at -80 C in cryovials containing 1.0 mL of brain heart infusion broth (Difco, Sparks, MD) with 20% glycerol.

DNA extraction and bacterial subtyping. Genomic DNA was extracted using Qiagen DNeasy kit (Qiagen, Valencia, CA) as described previously (Smith *et al.*, 2004). PCR was used to differentiate between *Campylobacter* species using species-specific primers, *hip* for *C. jejuni* and *ceuE* for *C. coli,* as described (Smith *et al.*, 2004). PFGE was conducted using *Sma*I (New England Biolabs) according to the PulseNet protocol (http://www.cdc.gov/PULSENET/protocols.htm), with some minor adjustment as described previously (D'Lima *et al.*, 2007). *Fla* typing was conducted with *flaA*-specific primers (Operon, Chicago, IL) as described (Smith *et al.*, 2004). Tagged image file format (TIFF) from PFGE and *fla* typing were analyzed by Bionumerics version 4.6, Applied Maths, Saint-Marten, Belgium). An optimization of 1.5% and a position tolerance of 1.5% were applied in PFGE analysis. For *fla* typing, optimization at 2.0% and position tolerance at 2.3% were used. In both methods, Dice similarity coefficient were calculated based on pairwise comparison of the DNA profiles obtained and the matrix of coefficient was used to generate dendograms based on the unweighted pair group method using arithmetic average (UPGMA).

For MLST, primer sets for 7 housekeeping genes (*aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt* and *uncA*) were used and PCR analysis was performed as described (Millet *et al.*, 2006), with minor modification (95°C for 45 s, 53°C for 45 s, and 72°C for 2 min, over 32 cycles) as described (Gu *et al.*, 2009). The first 8 amplicons were sequenced at the Genome Research Laboratory at North Carolina State University, Raleigh, and also at Genwiz Laboratory

(Genewiz, Inc., NJ). Another 8 more isolates were sequenced and identified at USDA-ARS laboratory, Albany, CA. Sequence type (ST) identification was done using the *C. jejuni/C. coli* MLST database available on the internet at http://pubmlst.org/campylobacter.

Minimum inhibitory concentration (MIC) determinations. MIC was determined for the following seven antimicrobial agents: ampicilin, ciprofloxacin, erythromycin, kanamycin, nalidixic acid, streptomycin, and tetracycline. MIC of erythromycin, ciprofloxacin, and tetracycline was determined as described previously (Lee *et al.*, 2005) with agar dilution method following the Clinical and Laboratory Standard Institute (CSLI) guidelines. Testing for MIC of ampicilin, kanamycin, streptomycin, and nalidixic acid was performed using the agar dilution method as described previously (Luangtongkum *et al.*, 2006). Testing range for all antimicrobials was from 0.5 to 256 µg/ml. *C. jejuni* ATCC 33560 (purchased from the American Type Culture Collection, Manassas, VA) was used as the quality control organism. MIC was defined as the lowest concentration of an antimicrobial agent that inhibited bacterial growth on the plates. The resistance breakpoints for MIC tests were as described previously (Luangtongkum *et al.*, 2006; Gu *et al*., 2009), except for streptomycin, where the resistant breakpoint was that used for Enterobacteriaceae, as recommended by CSLI (CSLI, 2007).

Statistic analysis. The antimicrobial resistance data to ciprofloxacin, nalidixic acid, and tetracycline were analyzed using the Spearman correlation coefficient to determine the linear association between the (rank of) concentrations required to inhibit isolates under pairs of antimicrobial agents. The computation of exact 95% confidence limits for each resistance probability (among a population of isolates) corresponding to different combinations of

antimicrobial agent and concentration was used. The variability in resistance across companies for all antimicrobial agents was analyzed using the nonparametric method one-way analysis of variance ("Kruskal-Wallis" test) for equal distribution of required concentration of agent to kill *Campylobacter* across the five companies.

2.4 RESULTS

A total of 152 MDR *C. coli* isolates were tested for MIC. This represents 26.4% (152/575) of the total number of MDR *C. coli* isolated by our laboratory between 2006 and 2009 (Figure 2.1). These isolates were from five different companies (integrators) with company A, C, and D accounting for more than 90% of the isolates (Figure 2.2). All isolates had been designated as MDR based on their resistance to 7 antimicrobial agents: ampicilin at 100 μg/ml, ciprofloxacin at 5 μg/ml, erythromycin at 15 μg/ml, kanamycin at 30 μg/ml, nalidixic acid at 30 μg/ml, streptomycin at 10 μg/ml, and tetracycline at 30 μg/ml.

MIC for antimicrobial agents. The MIC distribution and resistance rates for the 152 MDR *C. coli* isolates is presented in Table 2.2 and the results from individual isolates are presented in Table 2.3. Prevalence of resistance to individual antimicrobials was assessed and we found that the majority of the isolates were highly resistant (>250 μg/ml) to ampicilin (92.8%), erythromycin (99.3%), kanamycin (98.7%), and streptomycin (99.3%).

In the case of tetracycline, all but one of the isolates were able to grow beyond the resistance breakpoint level of 16 μg/ml; the exception was one isolate (SC2916) from company A. We identified 5 isolates that were not inhibited at 256 μg/ml of tetracycline.

These highly resistant isolates were all isolated in 2009 from company A (SC2680) and company B (SC2726, SC2732, SC2758, and SC2767).

The majority of the isolates were inhibited at 64 μg/ml (n=92/152) of nalidixic acid. Only one isolate (SC2893) was inhibited at 16 μg/ml, while the remainder were inhibited at higher nalidixic acid concentrations; 5.8% at 32 μg/ml, 60.9% at 64 μg/ml, and 32.7% at 128 μg/ml. The majority of the isolates were inhibited at 32 ug/ml (n=111/152) of ciprofloxacin (Table 2.2).

We also identified 33 isolates that showed relatively low resistance to multiple antimicrobial agents (Table 2.4). Most of these isolates were inhibited at the lower range of antimicrobial concentration, for example at 4 – 16 μg/ml of ciprofloxacin, at 16 to 32 μg/ml of nalidixic acid, and at 16 to 64 μg/ml of tetracycline. Isolates that inhibited at 128 and 256 μg/ml of ampicilin, erythromycin, kanamycin, and streptomycine were also included in this list. We observed that some isolates were less resistant to more than one type of antimicrobial agent. For instance, three isolates (SC2893, SC2909, and SC2937) that were inhibited by ciprofloxacin at 4 μg/ml were also inhibited to nalidixic acid at either 64 or 32 μg/ml. Another example, the SC2916 was inhibited at 16 μg/ml tetracycline and 8 μg/ml ciprofloxacin.

Tests with tetracycline, nalidixic acid, and ciprofloxacin produced a wide range of MIC among the MDR *C. coli* isolates. The MICs for tetracycline ranged from 16 to >256 μg/ml with the majority of the isolates (81/152) inhibited at 256 μg/ml (Table 2.2). For the antimicrobials in the fluroquinolone class, nalidixic acid and ciprofloxacin, the MICs ranged from 16 to 128 μg/ml for nalidixic acid, and from 4 to 64 μg/ml for ciprofloxacin (Table 2.2).

Using the Spearman correlation coefficient we also determined whether isolates resistant to high concentrations of one antimicrobial agent tended to be resistant to high concentrations of other antimicrobials agents. We found that there was liner correlation between ciprofloxacin and nalidixic acid ($p = 0.0221$), and between ciprofloxacin and tetracycline ($p < 0.0001$). This statistical analysis suggested that our MDR *C. coli* isolates have the tendency to produce the same pattern of antimicrobial resistance to ciprofloxacin and nalidixic acid, and also to ciprofloxacin and tetracycline. Weak correlation between levels of resistance to tetracycline and nalidixic acid ($p = 0.0637$) suggests that the resistance to tetracycline and nalidixic acid would be likely to exist but further clarification needs with more data.

We also examined the company effect on resistance to tetracycline, ciprofloxacin and nalidixic acid. Using the nonparametric one-way analysis of variance ("Kruskal-Wallis" test) we found evidence of significant company effect in resistance to ciprofloxacin ($p = 0.002$) and nalidixic acid ($p < 0.001$), but not for the resistance to tetracycline ($p = 0.3386$).

Fla typing and PFGE comparison. All of the isolates were typed with *fla* typing and PFGE. We were able to determine the PFGE profiles of all 152 isolates and the *fla* types of 150. Despite repeated efforts, we were unable to get good *fla* profiles of isolates SC1123 and SC2343. Using the BioNumerics software, 12 and 42 different genotype patterns were identified with *fla* and PFGE typing, respectively.

***Fla* typing.** The size of *flaA* in *C. coli* is about 1.7 kb. Digestion with the enzyme *Dde*I resulted in 5 to 7 DNA fragments with sizes ranging from 80 to 800 bp (Figure 2.3). The dendrogram of the *fla* types obtained from 150 isolates is shown in Figure 2.5. At

similarity ≥60.4%, we identified three major clusters; F1, F2, and F3. Type IDs was assigned, designated *a* to *l*. Based on similarity ≥99%, we identified 12 *fla* types, of which two were unique and 10 were shared by at least two isolates (Table 2.5 and Table 2.6). Company A has the most diverse *fla* genotype with 10 *fla* types (eight were shared and two were unique) followed by company D with eight *fla* types (six shared and two unique). Company B, C, and E has 3, 5 and 2 *fla* types, respectively (Table 2.5).

The predominant cluster was cluster F2 (consist of *fla* types *e* – *j*), which accounted for 118 (77.3%) of the total isolates, while cluster F1 (consist of *fla* types *a* – *d*) and cluster F3 (consist of *fla* types *k* and *l*) only have 18 and 14 isolates, respectively. Only cluster F2 has isolates from all 5 companies from 2006 to 2009. Both cluster F1 and cluster F3 has isolates only from companies A, C and D.

We identified two isolates (SC1835 and SC1743) with unique *fla* types. *Fla* types *b* and *c* were found only in isolates from company A, and *fla* types *d* and *h* were found only from isolates company D. Eight *fla* types (*a*, *e*, *f*, *g*, *i*, *j*, *h* and *l*) were shared by isolates from at least two companies.

Fla types *b* (n=1), *c* (n=2), *d* (n=1) and *g* (n=3) were found among isolates from 2007 while *fla* type *i* (n=5) was detected in isolates from 2009. The two most predominant *fla* types were type *e* (44 isolates) and type *f* (57 isolates) which accounted for 101 (67%) of the total MDR *C. coli* isolates that we tested. *Fla* types *e* and *f* were prevalence from 2006 to 2009. Only *fla* type *e* that can be found in all five companies in operating in this region.

PFGE. Genomic DNA from all 152 MDR *C. coli* isolates was digested with *Sma*I, producing between 6 and 10 DNA fragments with sizes ranging from 40 – 420 kb (Figure

2.4). The dendrogram of 45 different PFGE patterns identified from MDR *C. coli* isolates is shown in Figure 2.6. PFGE patterns with >97% similarity were considered identical, reducing the total number of PFGE profiles to 42. Each PFGE profile was identified with a number from 1 to 42.

The number of PFGE types identified in isolates from different companies is shown in Table 2.7. We identified 42 types of PFGE patterns where 21 were distinct and 21 were shared by 2 to 20 isolates. Company D has the most PFGE profiles with 25 profiles, followed by company A with 24 profiles, and company C with 18 profiles. Company B and E only produced 5 and 3 PFGE profiles, respectively. The number of PFGE profiles for each company was tabulated in Table 2.8. PFGE profile type 8 and 18 were the only two that were shared by isolates from 4 different companies. We found 9 PFGE profiles were shared by isolates from company A, C and D. A total of 14 PFGE profiles were shared by isolates from company A and D, and 10 PFGE profiles were shared by isolates from company A and C. Isolates from company C and D shared 11 PFGE profiles.

Distinct PFGE profiles were identified in company A, C, D and E. Company A and C both have 6, company D has 7, and company E only has 1 distinct PFGE profile. No distinct PFGE profile was identified in isolates from company B (Table 2.8).

We identified 4 major clusters (P1 to P4) that have similarity in PFGE pattern of ≥74.8%. Clusters P1, P2, P3, and P4 have 35, 47, 37 and 28 isolates, respectively. PFGE types 17, 35, and 42 represented unique PFGE patterns. Even though PFGE type 35 has 3 different isolates, we considered this profile unique because the PFGE pattern only had

70.3% similarity with the other PFGE patterns identified in this study, the dendrogram also showed this profile were significantly different.

Based on the PFGE and *fla* profiles, we identified 77 different genotypes and the majority (n=48) of them were unique (Figure 2.7). The genotype for each of the 152 MDR *C. coli* based on the *fla* and PFGE profiles were tabulated in Table 2.9. All the 21 distinct PFGE profiles does not have unique *fla* types as shown in Table 2.10. On the same note, the two distinct *fla* types (type *b* and *d*) also did not have unique PFGE profiles.

MLST. The 16 isolates were typed by MLST to represent the isolates from major PFGE-based clusters and also some from some of the unique PFGE profiles in the MDR *C. coli* isolates. We found four turkey-specific STs: ST889, ST1101, ST1126 and ST1149, and one ST5131 was newly identified in this study (Table 2.11). Isolates with the same ST were grouped in the same PFGE-based clusters at ≥74.8% for ST1149, ≥79.4% for ST1126, and ≥78% and ≥84.7% for ST1101 (Figure 2.6). Most of the STs having close similarity in PFGE pattern as shown in isolates SC2073 and SC2024 (ST1101), SC1585, SC2818 and SC2211 (ST1101), SC1994 and SC1742 (ST1126), and SC2002 and SC1680 (ST1149). Based on the number of ST identified and the distribution of isolates by PFGE pattern, we would suggest that ST1101 was the most predominant clonal group currently colonizing the turkey flocks in this region.

2.5 DISCUSSION

Antimicrobial susceptibility test. All of our MDR *C. coli* isolates were from commercially grown turkeys managed by 5 different companies or integrators. This study

was conducted to monitor and to compare the prevalence of multidrug resistance in *C. coli* from conventional turkey production between 2006 and 2009.

The selection of resistance breakpoints in this study was similar to Gu *et al*. (2009) and Luangtongkum *et al*. (2006) who also investigated the prevalence of antimicrobial resistance to *Campylobacter* in turkeys. The resistance breakpoints used in this study for ampicillin, ciprofloxacin, nalidixic acid, and tetracycline were similar to the other studies with isolates from chickens and turkeys (Luber *et al*., 2003; Lutgen *et al*., 2009, Ge *et al*., 2003) as well as isolates from cattle (Englen *et al*., 2005). In the study by Luber *et al*. (2003) using the agar dilution method, the breakpoints for ampicillin, ciprofloxacin and tetracycline were similar to those of National Antimicrobial Resistance Monitoring System (NARMS), except for erythromycin which was ≥8 μg/ml, lower than the ≥32 μg/ml in NARMS.

The study by Englen *et al*. (2005) with *Campylobacter* isolated from cattle employed the E-test (AB-Biodisk, Piscataway, NJ, USA) that was adopted by NARMS for susceptibility testing of *Campylobacter*. The selected resistance breakpoints were: 4 μg/ml for ciprofloxacin, ≥32 μg/ml for erythromycin, ≥32 μg/ml for nalidixic acid, and ≥16 μg/ml for tetracycline, all of which were similar to those of NARMS except for nalidixic acid. There were also variations in selecting resistance breakpoints for erythromycin and nalidixic acid. NARMS and Lutgen *et al*. (2009) used >32 μg/ml as the resistance breakpoint for erythromycin. Furthermore, NARMS selected >64 μg/ml as breakpoint for nalidixic acid resistance.

Our MIC results differed from those reported by NARMS (http://www.cdc.gov/narms/annual/2008/NARMS_2008_Annual_Report.pdf). According to

this report, the highest MIC for *C. coli* isolates from human clinical samples was 64 μg/ml for ciprofloxacin, and 128 μg/ml for erythromycin, nalidixic acid, and tetracycline. MIC for ampicillin, streptomycin, and kanamycin were not tested by NARMS. Our MDR *C. coli* isolates from turkey also produced the highest MIC similar with NARMS in ciprofloxacin and nalidixic acid test. However, in test against erythromycin and tetracycline, a large number of our isolates produced higher MIC level to erythromycin and tetracycline (Table 2.2).

Most of the MDR *C. coli* isolates from turkeys were highly resistant to ampicillin, streptomycin, kanamycin, and erythromycin. Our results were similar with those from a previous study conducted by Luangtongkum *et al*. (2006) which also found that the majority of their *C. coli* isolates were not inhibited at 128 μg/ml for same type of antimicrobial agents.

In previous results from our laboratory (Gu *et al*., 2009), the percentage of *C. jejuni* that were not inhibited at 256 μg/ml of ampicillin, streptomycin and kanamycin was also high. In contrast, all *C. jejuni* isolates from turkeys from the same region were inhibited by erythromycin at 4 μg/ml. This finding is in accordance to the previous work by Lutgen *et al*. (2009) on *Campylobacter* isolates from processed turkey where the frequency of resistance to ciprofloxacin and erythromycin were higher in *C. coli* than in *C. jejuni*. Zhao *et al*. (2010) also showed in their study that in comparison with the *C. jejuni*, the *C. coli* isolates from chicken breast were more resistant to all types of antimicrobial agents, except tetracycline.

The susceptibility to fluoroquinolones (nalidixic acid and ciprofloxacin) was still significant, despite the fact that FDA banned the use of these drugs for veterinary purposes in

September 2005 (FDA, 2005). Antimicrobials were used in animal husbandry to prevent and to treat disease and also used as growth promoters (McEwen and Federoka-Cray, 2002).

Nalidixic acid and ciprofloxacin are both from the same class of fluoroquinolones and have similar mechanisms to inhibit *Campylobacter*. It is not surprising to see that *Campylobacter* would have similar resistance to both drugs and in our study we were able to show the significant correlation between these two antibiotics in inhibiting our MDR *C. coli* isolates. The correlation between resistance levels to tetracycline and to fluoroquinolones was also determined.

Tetracycline is commonly used in turkey farming (Chopra and Roberts, 2001) and fluoroquinolones were only relatively recently (September 2005) withdrawn from the poultry industry (FDA, 2005). It is been suggested that the level of antimicrobial resistance could be reduced when the selective pressure has been removed (Aarestrup, 2005), however we suggest that the reduction in resistance will only be significant several years after the ban. The mechanisms of inhibition and resistance to tetracycline were very different than of the fluoroquinolones group (Luangtongkum *et al*., 2009). The weak correlation between tetracycline and nalidixic acid would suggest that this could be due to various differences between these two classes of drugs.

C. coli only represents up to 15% of total human infections but shows high resistance to multiple antimicrobials, including those that are important for disease treatment. A recent report by NARMS (CDC, 2010) also showed that most of the *Campylobacter* isolates were recovered from poultry products. Further study is required to monitor the susceptibility of *Campylobacter* to the fluoroquinolones class of drugs.

Friedman *et al*. (2000) reported that before the introduction of fluoroquinolones for human use in the United States in 1986, there were no fluoroquinolone-resistant *Campylobacter* strains found among human isolates. The resistant strains only started to appear one year after the introduction of the drug for human use and the rate increased significantly when the FDA approved its use in the poultry industry (Altekruse and Tollefson, 2003).

We have shown that MDR *C. coli* in turkey production were highly resistant to erythromycin and the level is much higher than reported by NARMS. The recent study by Logue *et al*. (2010) also showed that a majority of the isolates displayed high resistance (>256 μg/ml) to erythromycin. Transformation to erythromycin resistance in *C. coli* from turkeys has been reported, and resistance in the transformants is stable even in the absence of the antibiotic (Kim *et al*., 2006).

The correlation between macrolide resistance and the treatment regime applied in the facility, especially during the grow-out phase has been demonstrated recently (Logue *et al*., 2010). Studies by McEwen and Federoka-Cray (2002) have shown that resistance to some antibiotics can be reversed when the use of that antibiotic is discontinued. We believe that it would take a long time for the bacteria to reduce resistance to these antibiotics. Human involvement, animal, plant and environmental bacteria populations could contribute to the incidence of antibiotic resistance in pathogens (Mazel and Davies, 1999).

Distribution of the MDR C. coli isolates. We identified five types of STs from the 16 isolates that we tested, and four of them, ST889, ST1101, ST1126, and ST1149, were turkey-specific STs as specified in Miller *et al*. (2006). The ST5131 was newly identified in

this study. Except the ST5131, all other STs were previously identified in turkey flocks in this region (D'lima et al., 2007).

Since we only have 10 and 4 isolates from company B and E, respectively, we believe that the number was not sufficient to conclude the clonal population currently prevalence in both companies. More isolates needed to be tested from these companies.

There could be more STs currently present among the MDR *C. coli* in turkey flocks. However, based on the assumption that MLST results have a strong correlation with PFGE (Lee *et al*., 2005), we suggested that the findings could give the currently prevalent STs in this industry.

We only found 12 different *fla* types from 152 isolates that we tested. This was much less than the 42 PFGE types identified from the same isolates. We have selected the enzyme *Dde*I that according to Wassenaar and Newell (2000) should produce the best discriminatory power in *fla* typing. This enzyme was preferred due to its ease to perform, was less expensive, and its application to high throughput analysis. We found that 101 (67%) of our isolates belong to two types of *fla* (type *e* and *f*). Using *fla* typing alone we would not be able to differentiate more than half of our isolates.

Our study has shown a decrease in diversity of *fla* types in MDR *C. coli*. We only identified 12 *fla* types from 150 MDR *C. coli* isolates compared to the previous report from our laboratory that found 15 *fla* type from only 68 strains isolated from 2002 to 2004 (D'lima *et al*., 2007). The decrease in diversity could be due to prevalence of certain types of strains that currently colonize turkey flocks in this region.

The disadvantage of using *flaA* gene to distinguished closely related strains has been reported previously (Dingle *et al*., 2005). According to this report, *flaA* is highly variable due to the numerous exchanges that occurred between *C. coli* and *C. jejuni*. However, *fla* typing could still provide additional information that could increase the discriminatory power when used in combination with other genotyping methods (Wassenaar and Newell, 2000; Dingle *et al*., 2005).

*Fla*A gene typing cannot be used for species identification because the gene is highly variable and recombination between *C. coli* and *C. jejuni* has frequently occurred (Dingle *et al*., 2005). One study showed that recombination between *fla*A and *flaB* might occur at an unknown rate and this has reduced the value of flagellin-genotyping schemes (Wassenaar *et al*, 1998). However, this technique still can produce additional value, especially when used with other genotyping methods, to distinguished closely related *Campylobacter* strains (Dingle *et al*., 2005).

Some studies have shown that the result from PFGE and *fla* typing is not consistent and thus not suitable for long-term investigation. It is been suggested that genomic rearrangements have resulted in diversity in PFGE patterns (Wassenaar *et al*., 1998; Hanninen *et al*., 1999), and this could occur during intestinal colonization (Hanninen *et al*., 1999). PFGE provides more discriminatory power than *fla* typing, but both methods were complementary in genotyping *C. coli*. (Ge *et al*., 2006; Thakur *et al*., 2009).

We found that restriction enzyme *Sma*I gave us good resolution and the DNA fragments were separated and easily identified. We were able to generate PFGE profiles using *Sma*I from all 152 *C. coli* isolates, suggesting the suitability of this enzyme for studies

of *C. coli* turkey isolates. We observed some good correlation of PFGE profiles with the antimicrobial resistance. For instance, all 5 isolates that were highly resistant to tetracycline (≥256 μg/ml) had closely-related PFGE patterns of ≥95% and 4 of them have identical PFGE patterns. Our findings are similar to Zhao *et al*., (2010). Other studies also demonstrated that most of the *Campylobacter* isolates that have identical *fla*-PFGE types also have the same antimicrobial resistance profiles (Lutgen *et al*., 2009; Ge *at al*., 2003). We noticed that some PFGE profiles were obtained from isolates from more than one company and in different years between 2006 and 2009, suggesting that dissemination of certain types of strain in the turkey industry in this region.

Our findings also show the importance of using more than one type of genotyping method to further characterize MDR *C. coli*. The genomic diversification among MDR isolates can be further characterized with the combination of *fla* and PFGE typing. We were able to identify 77 different genotypes among these 152 isolates. This present observation could provide a good source of reference for source tracking for animal or human isolates.

2.6 CONCLUSION

The level of resistance among the MDR *C. coli* isolated from commercially grown turkey in this region is still high and we have shown the evidence of correlation in resistance within the fluoroquinolones (nalidixic acid and ciprofloxacin) and also the correlation between fluoroquinolones and tetracycline. We also demonstrated that there was significant difference in levels of resistance to fluoroquinolones for isolates coming from different companies, suggesting that the different regimes applied by different companies may play a

role in the level of fluoroquinolones resistance. We have shown the prevalence of turkey-specific STs colonizing the turkey production in this region and this information could be used as reference for future investigation to determine the extent to which *Campylobacter* from turkeys contributes to human infection. The results from our genotyping with PFGE, *fla* and MLST can be used to compare with isolates from humans in this region. Further studies are needed to determine whether the level of resistance to fluoroquinolones can be reduced after the approval by FDA was withdrawn in 2005.

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Table 2.1. List of 152 MDR *C. coli* isolates investigated in this study.

No.	Isolate	Received	Company	Age (day)
1	SC1473	12/13/2006	A	30 d
2	SC1481	12/13/2006	A	27 d
3	SC1482	12/13/2006	A	27 d
4	SC1499	12/13/2006	A	48 d
5	SC1501	12/13/2006	A	Not available
6	SC1512	12/13/2006	A	22 d
7	SC1520	12/13/2006	A	29 d
8	SC1491	12/13/2006	A	Not available
9	SC1494	12/13/2006	A	Not available
10	SC1436	12/13/2006	C	30 d
11	SC1444	12/13/2006	C	24 d
12	SC1445	12/13/2006	C	24 d
13	SC1454	12/13/2006	C	21 d
14	SC1458	12/13/2006	C	40 d
15	SC1460	12/13/2006	C	40 d
16	SC1472	12/13/2006	C	24 d
17	SC1443	12/13/2006	C	24 d
18	SC1558	3/6/2007	D	25 d
19	SC1571	3/6/2007	D	14 d
20	SC1572	3/6/2007	D	14 d
21	SC1577	3/6/2007	D	25 d
22	SC1581E	3/6/2007	D	39 d
23	SC1583E	3/6/2007	D	39 d
24	SC1585	3/6/2007	D	17 d
25	SC1591	3/6/2007	D	29 d
26	SC1598	3/6/2007	D	21 d
27	SC1572E	3/6/2007	D	14 d
28	SC1583	3/6/2007	D	39 d
29	SC1599	3/6/2007	D	21 d
30	SC1649	3/7/2007	A	44 d
31	SC1672	3/7/2007	A	28 d
32	SC1677	3/7/2007	A	35 d
33	SC1683	3/7/2007	A	35 d

Table 2.1. Continued.

No.	Isolate	Received	Company	Age (day)
34	SC1683E	3/7/2007	A	35 d
35	SC1685E	3/7/2007	A	47 d
36	SC1693	3/7/2007	A	51 d
37	SC1679	3/7/2007	A	35 d
38	SC1680	3/7/2007	A	35 d
39	SC1617	3/7/2007	C	75 d
40	SC1634	3/7/2007	C	91 d
41	SC1727	6/26/2007	D	11 d
42	SC1730	6/26/2007	D	11 d
43	SC1733	6/26/2007	D	35 d
44	SC1737	6/26/2007	D	21 d
45	SC1742	6/26/2007	D	39 d
46	SC1743	6/26/2007	D	39 d
47	SC1745-3	6/26/2007	D	24 d
48	SC1747	6/26/2007	D	24 d
49	SC1756	6/26/2007	D	28 d
50	SC1761	6/26/2007	D	28 d
51	SC1765	6/26/2007	D	24 d
52	SC1770	6/26/2007	D	20 d
53	SC1775	6/26/2007	D	13 d
54	SC1779	6/26/2007	D	14 d
55	SC1783	6/26/2007	D	32 d
56	SC1786-3	6/26/2007	D	34 d
57	SC1745	6/26/2007	D	24 d
58	SC1788	6/26/2007	D	34 d
59	SC1836E	6/27/2007	A	42 d
60	SC1821	6/27/2007	A	63 d
61	SC1822	6/27/2007	A	63 d
62	SC1826	6/27/2007	A	26 d
63	SC1832	6/27/2007	A	50 d
64	SC1834	6/27/2007	A	42 d
65	SC1834E-3	6/27/2007	A	42 d
66	SC1835	6/27/2007	A	42 d
67	SC1823	6/27/2007	A	63 d
68	SC1789	6/27/2007	C	43 d

Table 2.1. Continued.

No.	Isolate	Received	Company	Age (day)
69	SC1790	6/27/2007	C	43 d
70	SC1799	6/27/2007	C	10 d
71	SC1799E-3	6/27/2007	C	10 d
72	SC1870	6/28/2007	B	33 d
73	SC1943	1/15/2008	D	24 d
74	SC1948	1/15/2008	D	14 d
75	SC1954	1/15/2008	D	26 d
76	SC1958	1/15/2008	D	20 d
77	SC1961	1/15/2008	D	20 d
78	SC1969	1/15/2008	D	28 d
79	SC1970	1/15/2008	D	28 d
80	SC1978	1/15/2008	D	39 d
81	SC1994	1/15/2008	D	32 d
82	SC2047	1/16/2008	A	35 d
83	SC2048	1/16/2008	A	35 d
84	SC2057	1/16/2008	A	24 d
85	SC2062	1/16/2008	A	41 d
86	SC2063	1/16/2008	A	41 d
87	SC2066	1/16/2008	A	43 d
88	SC2068	1/16/2008	A	43 d
89	SC2073	1/16/2008	A	60 d
90	SC2004	1/16/2008	C	59 d
91	SC2007	1/16/2008	C	56 d
92	SC2009	1/16/2008	C	37 d
93	SC2010	1/16/2008	C	37 d
94	SC2024	1/16/2008	C	12 d
95	SC2094	1/17/2008	B	33 d
96	SC2111	5/20/2008	D	36 d
97	SC2114	5/20/2008	D	18 d
98	SC2135	5/20/2008	D	24 d
99	SC2153	5/20/2008	D	35 d
100	SC2162	5/20/2008	D	32 d
101	SC2163	5/20/2008	D	32 d
102	SC2211	5/21/2008	A	40 d
103	SC2213	5/21/2008	A	36 d

Table 2.1. Continued.

No.	Isolate	Received	Company	Age (day)
104	SC2235	5/21/2008	A	28 d
105	SC2215	5/21/2008	A	36 d
106	SC2171	5/21/2008	C	25 d
107	SC2182	5/21/2008	C	17 d
108	SC2190	5/21/2008	C	29 d
109	SC2184	5/21/2008	C	35 d
110	SC2288	5/22/2008	B	25 d
111	SC2565	6/16/2009	D	15 d
112	SC2578	6/16/2009	D	25 d
113	SC2583	6/16/2009	D	25 d
114	SC2595	6/16/2009	D	28 d
115	SC2605	6/16/2009	D	18 d
116	SC2623	6/17/2009	C	29 d
117	SC2633	6/17/2009	C	32 d
118	SC2640	6/17/2009	C	28 d
119	SC2642	6/17/2009	A	11 d
120	SC2648	6/17/2009	A	15 d
121	SC2651	6/17/2009	A	23 d
122	SC2656	6/17/2009	A	42 d
123	SC2659	6/17/2009	A	28 d
124	SC2661	6/17/2009	A	26 d
125	SC2671	6/17/2009	A	40 d
126	SC2675	6/17/2009	A	39 d
127	SC2680	6/17/2009	A	46 d
128	SC2682	6/24/2009	E	29 d
129	SC2688	6/24/2009	E	23 d
130	SC2722	6/25/2009	B	29 d
131	SC2726	6/25/2009	B	40 d
132	SC2732	6/25/2009	B	26 d
133	SC2753	6/25/2009	B	34 d
134	SC2758	6/25/2009	B	33 d
135	SC2767	6/25/2009	B	30 d
136	SC2780	12/1/2009	D	13 d
137	SC2800	12/1/2009	D	27 d
138	SC2818	12/1/2009	D	20 d

Table 2.1. Continued.

No.	Isolate	Received	Company	Age (day)
139	SC2835	12/1/2009	D	29 d
140	SC2843	12/2/2009	C	28 d
141	SC2849	12/2/2009	C	30 d
142	SC2893	12/2/2009	C	36 d
143	SC2895	12/2/2009	A	29 d
144	SC2909	12/2/2009	A	57 d
145	SC2916	12/2/2009	A	34 d
146	SC2923	12/2/2009	A	21 d
147	SC2924	12/2/2009	A	39 d
148	SC2937	12/2/2009	A	49 d
149	SC2944	12/2/2009	A	25 d
150	SC2984	12/3/2009	B	35 d
151	SC3018	12/9/2009	E	35 d
152	SC3021	12/9/2009	E	28 d

Table 2.2. The MIC distribution and resistance rates for 152 MDR *C. coli* isolated from turkeys from five different companies between 2006 and 2009.

Antimicrobial agent	95% CI[a] (Lowest obs.) (Highest obs.)	Number of isolates inhibited at antimicrobials concentration (µg/ml) of:										
		0.5	1	2	4	8	16	32	64	128	256	>256
Ampicilin	(0.00-0.02)[b] (0.04-0.13)	0	0	0	0	0	0	0	0	0	11	141
Erythromycin	(0.00-0.02)[b] (0.96-1.0)	0	0	0	0	0	0	0	0	0	1	151
Kanamycin	(0.00-0.02)[b] (0.95-1.0)	0	0	0	0	0	0	0	0	1	1	150
Tetracycline	(0.00-0.02)[b] (0.45-0.61)	0	0	0	0	0	1	3	14	48	81	5
Streptomycin	(0.00-0.02)[b] (0.96-1.0)	0	0	0	0	0	0	0	0	0	1	151
Nalidixic acid	(0.00-0.02)[b] (0.52-0.68)	0	0	0	0	0	1	9	92	50	0	0
Ciprofloxacin	(0.00-0.02)[b] (0.65-0.80)	0	0	0	3	2	10	111	26	0	0	0

[a] The range of 95% confidence interval (CI) for observations was calculated using the Clooper-Pearson exact method. The first (top) range is for the lowest observation and the second (bottom) range is for the highest observation for each antimicrobial agent.

[b] The 95% confidence interval for the MDR population proportion that would be inhibited by this combo is given by (0, 0.02) whenever zero (0) out of 152 isolates were inhibited in this study using the Clopper-Pearson exact method. The sampling correction factors were not included in any of these analyses, so quantifications of uncertainty and p-value for tests of equality may be on the conservative side.

Table 2.3. The Minimum inhibitory concentration (MIC) for multidrug resistance (MDR) *C. coli* isolated from turkey between 2006 and 2009.

No.	Isolate	Date received	Company	Age (day)	Amp[1]	Ery[2]	Kana[3]	Tetra[4]	Strepto[5]	Nali[6]	Cipro[7]
1	SC1473	12/13/2006	A	30 d	>256	>256	>256	256	>256	64	32
2	SC1481	12/13/2006	A	27 d	>256	>256	>256	256	>256	128	32
3	SC1482	12/13/2006	A	27 d	>256	>256	>256	256	>256	128	64
4	SC1499	12/13/2006	A	48 d	>256	>256	>256	256	>256	64	64
5	SC1501	12/13/2006	A	NA	>256	>256	>256	256	>256	64	32
6	SC1512	12/13/2006	A	22 d	>256	>256	>256	256	>256	64	32
7	SC1520	12/13/2006	A	29 d	>256	>256	>256	256	>256	64	64
8	SC1491	12/13/2006	A	NA	>256	>256	>256	128	>256	64	32
9	SC1494	12/13/2006	A	NA	>256	>256	>256	128	>256	64	32
10	SC1649	3/7/2007	A	44 d	>256	>256	>256	256	>256	128	64
11	SC1672	3/7/2007	A	28 d	>256	>256	>256	256	>256	128	64
12	SC1677	3/7/2007	A	35 d	>256	>256	>256	128	>256	32	32
13	SC1683	3/7/2007	A	35 d	>256	>256	>256	256	>256	32	32
14	SC1683E	3/7/2007	A	35 d	256	>256	>256	256	>256	32	32
15	SC1685E	3/7/2007	A	47 d	>256	>256	>256	256	>256	64	32
16	SC1693	3/7/2007	A	51 d	>256	>256	>256	256	>256	64	32
17	SC1679	3/7/2007	A	35 d	>256	>256	>256	64	>256	64	32
18	SC1680	3/7/2007	A	35 d	>256	>256	>256	64	>256	64	32
19	SC1836E	6/27/2007	A	42 d	256	>256	>256	128	>256	64	64
20	SC1821	6/27/2007	A	63 d	>256	>256	>256	128	>256	128	32
21	SC1822	6/27/2007	A	63 d	>256	>256	>256	128	>256	128	64
22	SC1826	6/27/2007	A	26 d	>256	>256	>256	256	>256	64	64
23	SC1832	6/27/2007	A	50 d	>256	>256	>256	128	>256	64	32
24	SC1834	6/27/2007	A	42 d	>256	>256	>256	128	>256	64	64
25	SC1834E-3	6/27/2007	A	42 d	>256	>256	>256	128	>256	64	32
26	SC1835	6/27/2007	A	42 d	256	>256	>256	64	>256	64	16
27	SC1823	6/27/2007	A	63 d	>256	>256	>256	256	>256	64	64
28	SC2047	1/16/2008	A	35 d	>256	>256	>256	128	>256	64	64
29	SC2048	1/16/2008	A	35 d	>256	>256	>256	128	>256	64	64
30	SC2057	1/16/2008	A	24 d	>256	>256	>256	128	>256	64	64
31	SC2062	1/16/2008	A	41 d	>256	>256	>256	256	>256	64	64
32	SC2063	1/16/2008	A	41 d	>256	>256	>256	256	>256	64	64
33	SC2066	1/16/2008	A	43 d	>256	>256	>256	256	>256	64	64
34	SC2068	1/16/2008	A	43 d	>256	>256	>256	256	>256	64	64
35	SC2073	1/16/2008	A	60 d	>256	>256	>256	256	>256	64	32
36	SC2211	5/21/2008	A	40 d	>256	>256	>256	256	>256	32	64

[1] Amp – ampicillin, [2] Ery – erythromycin, [3] Kana – kanamycin, [4] Tetra – tetracycline, [5] Strepto – streptomycin, [6] Nali – nalidixic acid, and [7] Cipro – ciprofloxacin

Table 2.3. Continued.

No.	Isolate	Date received	Company	Age (day)	Amp[1]	Ery[2]	Kana[3]	Tetra[4]	Strepto[5]	Nali[6]	Cipro[7]
37	SC2213	5/21/2008	A	36 d	>256	>256	>256	128	>256	128	32
38	SC2235	5/21/2008	A	28 d	>256	>256	>256	128	>256	128	32
39	SC2215	5/21/2008	A	36 d	>256	>256	>256	256	>256	128	32
40	SC1870	6/28/2007	B	33 d	>256	>256	>256	256	>256	128	64
41	SC2094	1/17/2008	B	33 d	>256	>256	>256	128	>256	128	32
42	SC2288	5/22/2008	B	25 d	>256	>256	>256	256	>256	128	64
43	SC1436	12/13/2006	C	30 d	>256	>256	>256	128	>256	64	32
44	SC1444	12/13/2006	C	24 d	>256	>256	>256	256	>256	128	32
45	SC1445	12/13/2006	C	24 d	>256	>256	>256	256	>256	128	32
46	SC1454	12/13/2006	C	21 d	>256	>256	>256	256	>256	128	32
47	SC1458	12/13/2006	C	40 d	>256	>256	>256	128	>256	128	16
48	SC1460	12/13/2006	C	40 d	>256	>256	>256	128	>256	128	32
49	SC1472	12/13/2006	C	24 d	>256	>256	>256	128	>256	128	32
50	SC1617	3/7/2007	C	75 d	>256	>256	>256	256	>256	128	32
51	SC1634	3/7/2007	C	91 d	>256	>256	>256	128	>256	128	32
52	SC1789	6/27/2007	C	43 d	>256	>256	>256	256	>256	128	32
53	SC1790	6/27/2007	C	43 d	>256	>256	>256	128	>256	128	32
54	SC1799	6/27/2007	C	10 d	>256	>256	>256	256	>256	128	32
55	SC1799E-3	6/27/2007	C	10 d	256	>256	>256	64	>256	128	32
56	SC2004	1/16/2008	C	59 d	256	>256	>256	64	>256	128	16
57	SC2007	1/16/2008	C	56 d	256	>256	>256	128	>256	128	32
58	SC2009	1/16/2008	C	37 d	256	>256	>256	128	>256	128	32
59	SC2010	1/16/2008	C	37 d	256	>256	>256	128	>256	128	32
60	SC2024	1/16/2008	C	12 d	256	>256	>256	128	>256	128	32
61	SC2171	5/21/2008	C	25 d	>256	>256	>256	128	>256	128	32
62	SC2182	5/21/2008	C	17 d	>256	>256	>256	256	>256	128	32
63	SC2190	5/21/2008	C	29 d	256	>256	>256	256	>256	128	32
64	SC2184	5/21/2008	C	35 d	256	>256	>256	64	>256	128	32
65	SC1443	12/13/2006	C	24 d	>256	>256	>256	128	>256	64	32
66	SC1558	3/6/2007	D	25 d	>256	>256	>256	256	>256	128	64
67	SC1571	3/6/2007	D	14 d	>256	>256	>256	256	>256	64	32
68	SC1572	3/6/2007	D	14 d	>256	>256	>256	128	>256	64	32
69	SC1577	3/6/2007	D	25 d	>256	>256	>256	128	>256	64	32
70	SC1581E	3/6/2007	D	39 d	>256	>256	>256	256	>256	32	16
71	SC1583E	3/6/2007	D	39 d	>256	>256	>256	128	>256	64	32
72	SC1585	3/6/2007	D	17 d	>256	>256	>256	128	>256	64	32
73	SC1591	3/6/2007	D	29 d	>256	>256	>256	128	>256	64	32
74	SC1598	3/6/2007	D	21 d	>256	>256	>256	64	>256	64	16
75	SC1572E	3/6/2007	D	14 d	>256	>256	>256	128	>256	64	32

[1] Amp – ampicillin, [2] Ery – erythromycin, [3] Kana – kanamycin, [4] Tetra – tetracycline, [5] Strepto – streptomycin, [6] Nali – nalidixic acid, & [7] Cipro – ciprofloxacin

Table 2.3. Continued.

No.	Isolate	Date received	Company	Age (day)	Amp[1]	Ery[2]	Kana[3]	Tetra[4]	Strepto[5]	Nali[6]	Cipro[7]
76	SC1583	3/6/2007	D	39 d	>256	>256	>256	128	>256	64	32
77	SC1599	3/6/2007	D	21 d	>256	>256	>256	64	>256	64	16
78	SC1727	6/26/2007	D	11 d	>256	>256	>256	256	>256	64	32
79	SC1730	6/26/2007	D	11 d	>256	>256	>256	256	>256	128	32
80	SC1733	6/26/2007	D	35 d	>256	>256	>256	256	>256	64	32
81	SC1737	6/26/2007	D	21 d	>256	>256	>256	128	>256	128	32
82	SC1742	6/26/2007	D	39 d	>256	>256	>256	256	>256	64	32
83	SC1743	6/26/2007	D	39 d	>256	>256	>256	256	>256	64	32
84	SC1745-3	6/26/2007	D	24 d	>256	>256	>256	256	>256	64	32
85	SC1747	6/26/2007	D	24 d	>256	>256	>256	256	>256	64	32
86	SC1756	6/26/2007	D	28 d	>256	>256	>256	256	>256	64	32
87	SC1761	6/26/2007	D	28 d	>256	>256	>256	256	>256	64	32
88	SC1765	6/26/2007	D	24 d	>256	>256	>256	128	>256	64	32
89	SC1770	6/26/2007	D	20 d	>256	>256	>256	256	>256	64	32
90	SC1775	6/26/2007	D	13 d	>256	>256	>256	256	>256	64	32
91	SC1779	6/26/2007	D	14 d	>256	>256	>256	128	>256	64	32
92	SC1783	6/26/2007	D	32 d	>256	>256	>256	256	>256	64	32
93	SC1786-3	6/26/2007	D	34 d	>256	>256	>256	256	>256	64	32
94	SC1745	6/26/2007	D	24 d	>256	>256	>256	256	>256	64	32
95	SC1788	6/26/2007	D	34 d	>256	>256	>256	128	>256	64	32
96	SC1943	1/15/2008	D	24 d	>256	>256	>256	128	>256	64	16
97	SC1948	1/15/2008	D	14 d	>256	>256	>256	128	>256	64	16
98	SC1954	1/15/2008	D	26 d	>256	>256	>256	64	>256	64	32
99	SC1958	1/15/2008	D	20 d	>256	>256	>256	64	>256	64	8
100	SC1961	1/15/2008	D	20 d	>256	>256	>256	128	>256	64	32
101	SC1969	1/15/2008	D	28 d	>256	>256	>256	128	>256	64	32
102	SC1970	1/15/2008	D	28 d	>256	>256	>256	256	>256	128	32
103	SC1978	1/15/2008	D	39 d	>256	>256	>256	256	>256	64	32
104	SC1994	1/15/2008	D	32 d	>256	>256	>256	256	>256	64	32
105	SC2111	5/20/2008	D	36 d	>256	>256	>256	64	>256	64	32
106	SC2114	5/20/2008	D	18 d	>256	>256	>256	256	>256	128	64
107	SC2135	5/20/2008	D	24 d	>256	>256	>256	256	>256	128	32
108	SC2153	5/20/2008	D	35 d	>256	>256	>256	256	>256	128	32
109	SC2162	5/20/2008	D	32 d	>256	>256	>256	256	>256	128	64
110	SC2163	5/20/2008	D	32 d	>256	>256	>256	256	>256	128	64
111	SC2565	6/16/2009	D	15 d	>256	>256	>256	256	>256	64	32
112	SC2578	6/16/2009	D	25 d	>256	>256	>256	256	>256	64	32
113	SC2583	6/16/2009	D	25 d	>256	>256	>256	256	>256	128	32

[1] Amp – ampicillin, [2] Ery – erythromycin, [3] Kana – kanamycin, [4] Tetra – tetracycline, [5] Strepto – streptomycin, [6] Nali – nalidixic acid, and [7] Cipro – ciprofloxacin

Table 2.3. Continued.

No.	Isolate	Date received	Company	Age (day)	Amp[1]	Ery[2]	Kana[3]	Tetra[4]	Strepto[5]	Nali[6]	Cipro[7]
114	SC2595	6/16/2009	D	28 d	>256	>256	>256	256	>256	64	32
115	SC2605	6/16/2009	D	18 d	>256	>256	>256	128	>256	64	16
116	SC2623	6/17/2009	C	29 d	>256	>256	>256	256	>256	64	32
117	SC2633	6/17/2009	C	32 d	>256	>256	>256	256	>256	64	32
118	SC2640	6/17/2009	C	28 d	>256	>256	>256	256	>256	64	64
119	SC2642	6/17/2009	A	11 d	>256	>256	>256	256	>256	64	32
120	SC2648	6/17/2009	A	15 d	>256	>256	>256	256	>256	64	64
121	SC2651	6/17/2009	A	23 d	>256	>256	>256	256	>256	128	32
122	SC2656	6/17/2009	A	42 d	>256	>256	>256	256	>256	64	32
123	SC2659	6/17/2009	A	28 d	>256	>256	>256	256	>256	64	32
124	SC2661	6/17/2009	A	26 d	>256	>256	>256	256	>256	128	32
125	SC2671	6/17/2009	A	40 d	>256	>256	>256	128	>256	64	32
126	SC2675	6/17/2009	A	39 d	>256	>256	>256	128	>256	64	32
127	SC2680	6/17/2009	A	46 d	>256	>256	>256	>256	>256	128	32
128	SC2682	6/24/2009	E	29 d	>256	>256	>256	256	>256	128	32
129	SC2688	6/24/2009	E	23 d	>256	>256	>256	256	>256	128	32
130	SC2722	6/25/2009	B	29 d	>256	>256	>256	128	>256	64	32
131	SC2726	6/25/2009	B	40 d	>256	>256	>256	>256	>256	64	32
132	SC2732	6/25/2009	B	26 d	>256	>256	>256	>256	>256	64	32
133	SC2753	6/25/2009	B	34 d	>256	>256	>256	256	>256	64	32
134	SC2758	6/25/2009	B	33 d	>256	>256	>256	>256	>256	128	64
135	SC2767	6/25/2009	B	30 d	>256	>256	>256	>256	>256	64	32
136	SC2780	12/1/2009	D	13 d	>256	>256	>256	256	>256	64	32
137	SC2800	12/1/2009	D	27 d	>256	>256	>256	256	>256	64	32
138	SC2818	12/1/2009	D	20 d	>256	>256	>256	256	>256	64	32
139	SC2835	12/1/2009	D	29 d	>256	>256	>256	128	>256	64	32
140	SC2843	12/2/2009	C	28 d	>256	>256	>256	256	>256	128	32
141	SC2849	12/2/2009	C	30 d	>256	>256	>256	256	>256	64	32
142	SC2893	12/2/2009	C	36 d	>256	>256	>256	64	256	16	4
143	SC2895	12/2/2009	A	29 d	>256	>256	>256	256	>256	64	32
144	SC2909	12/2/2009	A	57 d	>256	>256	128	32	>256	32	4
145	SC2916	12/2/2009	A	34 d	>256	>256	>256	16	>256	64	8
146	SC2923	12/2/2009	A	21 d	>256	>256	>256	32	>256	32	32
147	SC2924	12/2/2009	A	39 d	>256	>256	>256	64	>256	32	32
148	SC2937	12/2/2009	A	49 d	>256	>256	256	32	>256	32	4
149	SC2944	12/2/2009	A	25 d	>256	>256	>256	128	>256	64	32
150	SC2984	12/3/2009	B	35 d	>256	>256	>256	256	>256	64	32
151	SC3018	12/9/2009	E	35 d	>256	256	>256	64	>256	64	16
152	SC3021	12/9/2009	E	28 d	>256	>256	>256	256	>256	64	32

[1] Amp – ampicillin, [2] Ery – erythromycin, [3] Kana – kanamycin, [4] Tetra – tetracycline, [5] Strepto – streptomycin, [6] Nali – nalidixic acid, & [7] Cipro – ciprofloxacin

Table 2.4. The Minimum inhibitory concentration for MDR *C. coli* that were considered relatively less resistance to at least one type of antimicrobial agent.

No.	Isolate	Date received	Company	Age (day)	Amp[1]	Ery[2]	Kana[3]	Tetra[4]	Strepto[5]	Nali[6]	Cipro[7]
1	SC1677	3/7/2007	A	35 d	>256	>256	>256	128	>256	32	32
2	SC1683	3/7/2007	A	35 d	>256	>256	>256	256	>256	32	32
3	SC1683E	3/7/2007	A	35 d	256	>256	>256	256	>256	32	32
4	SC1679	3/7/2007	A	35 d	>256	>256	>256	64	>256	64	32
5	SC1680	3/7/2007	A	35 d	>256	>256	>256	64	>256	64	32
6	SC1836E	6/27/2007	A	42 d	256	>256	>256	128	>256	64	64
7	SC1835	6/27/2007	A	42 d	256	>256	>256	64	>256	64	16
8	SC2211	5/21/2008	A	40 d	>256	>256	>256	256	>256	32	64
9	SC1458	12/13/2006	C	40 d	>256	>256	>256	128	>256	128	16
10	SC1799E-3	6/27/2007	C	10 d	256	>256	>256	64	>256	128	32
11	SC2004	1/16/2008	C	59 d	256	>256	>256	64	>256	128	16
12	SC2007	1/16/2008	C	56 d	256	>256	>256	128	>256	128	32
13	SC2009	1/16/2008	C	37 d	256	>256	>256	128	>256	128	32
14	SC2010	1/16/2008	C	37 d	256	>256	>256	128	>256	128	32
15	SC2024	1/16/2008	C	12 d	256	>256	>256	128	>256	128	32
16	SC2190	5/21/2008	C	29 d	256	>256	>256	256	>256	128	32
17	SC2184	5/21/2008	C	35 d	256	>256	>256	64	>256	128	32
18	SC1581E	3/6/2007	D	39 d	>256	>256	>256	256	>256	32	16
19	SC1598	3/6/2007	D	21 d	>256	>256	>256	64	>256	64	16
20	SC1599	3/6/2007	D	21 d	>256	>256	>256	64	>256	64	16
21	SC1943	1/15/2008	D	24 d	>256	>256	>256	128	>256	64	16
22	SC1948	1/15/2008	D	14 d	>256	>256	>256	128	>256	64	16
23	SC1954	1/15/2008	D	26 d	>256	>256	>256	64	>256	64	32
24	SC1958	1/15/2008	D	20 d	>256	>256	>256	64	>256	64	8
25	SC2111	5/20/2008	D	36 d	>256	>256	>256	64	>256	64	32
26	SC2605	6/16/2009	D	18 d	>256	>256	>256	128	>256	64	16
27	SC2893	12/2/2009	C	36 d	>256	>256	>256	64	256	16	4
28	SC2909	12/2/2009	A	57 d	>256	>256	128	32	>256	32	4
29	SC2916	12/2/2009	A	34 d	>256	>256	>256	16	>256	64	8
30	SC2923	12/2/2009	A	21 d	>256	>256	>256	32	>256	32	32
31	SC2924	12/2/2009	A	39 d	>256	>256	>256	64	>256	32	32
32	SC2937	12/2/2009	A	49 d	>256	>256	256	32	>256	32	4
33	SC3018	12/9/2009	E	35 d	>256	256	>256	64	>256	64	16

[1] Amp – ampicillin, [2] Ery – erythromycin, [3] Kana – kanamycin, [4] Tetra – tetracycline, [5] Strepto – streptomycin, [6] Nali – nalidixic acid, and [7] Cipro – ciprofloxacin.

Table 2.5. The number of the distinct and shared *fla* types for MDR *C. coli* received from 5 different companies.

Company	*fla* type		Total
	Distinct	Shared	
A	1	9	10
B	0	3	3
C	0	5	5
D	1	7	8
E	0	2	2

Table 2.6. The distribution of the 12 *fla* types of MDR *C. coli* isolated from five companies.

***Fla* types**	**Companies**					**Total**
	A	B	C	D	E	
a	2	-	4	8	-	14
b	***1***	-	-	-	-	1
c	2	-	-	-	-	2
d	-	-	-	***1***	-	1
e [1]	15	3	10	13	3	44
f [2]	22	2	11	22	-	57
g	3	-	-	1	-	4
h	-	-	-	3	-	3
i	2	-	1	1	1	5
j	1	4	-	-	-	5
k	4	-	-	5	-	9
l	2	-	3	-	-	5
Total	54	9	29	54	4	150

1 Number in **bold** and *italic* indicate the distinct *fla* type.
[1] *fla* type *e* was the only type that was shared by isolates from five different companies.
[2] Type *f* was shared by 4 companies.

Table 2.7. The number of the distinct and shared PFGE profiles for MDR *C. coli* received from 5 different companies.

Company	PFGE profile		Total
	Distinct	Shared	
A	6	18	24
B	0	5	5
C	6	12	18
D	7	18	25
E	1	2	3

Table 2.8. The distribution of the 42 PFGE profiles identified in 152 MDR *C. coli* isolates from five different companies in 2006 to 2009.

PFGE types	**Companies**					**Total**[a]
	A	B	C	D	E	
1	1		1	1		3
2			***1***			1
3					***1***	1
4	***1***					1
5				3		3
6				***1***		1
7	1				2	3
8	2		1	2	1	6
9	1			1		2
10	***1***					1
11	2		1	2		5
12	1		2	1		4
13				***1***		1
14				***1***		1
15	***1***					1
16			***1***			1
17				1		1
18	5	1	6	8		20
19	***1***					1
20	6	6		3		15
21			1	1		2
22	***1***					1
23	1			1		2
24			***1***			1
25	2	1				3
26			***1***			1
27				***1***		1
28	1			2		3
29	3		1	9		13
30			***1***			1
31				***1***		1
32	3		1	2		6
33	7		4	1		12
34				***1***		1
35			1	2		3
36	2					2
37			***1***			1
38	2	1	3			6
39	5		1	5		11
40	4	1		2		7
41	***1***					1
42				***1***		1
Total[a]	55	10	29	54	4	152

1 - Number in **bold** and *italic* indicate the distinct PFGE profile.
[a] The total number of isolates

Table 2.9. Source and genotype properties of the MDR *C. coli* isolates from 5 companies.

No.	ID	Received	Company	Age	PFGE type	*fla* type	PFGE and *fla* genotype
1	SC1473	12/13/2006	A	30 d	20	*f*	45
2	SC1481	12/13/2006	A	27 d	8	*e*	30
3	SC1482	12/13/2006	A	27 d	8	*e*	30
4	SC1499	12/13/2006	A	48 d	18	*f*	44
5	SC1501	12/13/2006	A	-	20	*f*	45
6	SC1512	12/13/2006	A	22 d	20	*e*	51
7	SC1520	12/13/2006	A	29 d	20	*f*	45
8	SC1491	12/13/2006	A	-	29	*j*	62
9	SC1494	12/13/2006	A	-	40	*f*	12
10	SC1649	3/7/2007	A	44 d	15	*g*	26
11	SC1672	3/7/2007	A	28 d	29	*a*	4
12	SC1677	3/7/2007	A	35 d	40	*g*	13
13	SC1683	3/7/2007	A	35 d	25	*c*	63
14	SC1683E	3/7/2007	A	35 d	25	-	63
15	SC1685E	3/7/2007	A	47 d	18	*e*	42
16	SC1693	3/7/2007	A	51 d	32	*c*	10
17	SC1679	3/7/2007	A	35 d	40	*g*	13
18	SC1680	3/7/2007	A	35 d	40	*f*	12
19	SC1836E	6/27/2007	A	42 d	33	*f*	46
20	SC1821	6/27/2007	A	63 d	39	*f*	15
21	SC1822	6/27/2007	A	63 d	39	*f*	15
22	SC1826	6/27/2007	A	26 d	12	*f*	37
23	SC1832	6/27/2007	A	50 d	23	*f*	54
24	SC1834	6/27/2007	A	42 d	18	*f*	44
25	SC1834E-3	6/27/2007	A	42 d	18	*f*	44
26	SC1835	6/27/2007	A	42 d	39	*b*	18
27	SC1823	6/27/2007	A	63 d	18	*e*	42
28	SC2047	1/16/2008	A	35 d	33	*l*	73
29	SC2048	1/16/2008	A	35 d	39	*f*	15
30	SC2057	1/16/2008	A	24 d	33	*e*	49
31	SC2062	1/16/2008	A	41 d	36	*l*	67
32	SC2063	1/16/2008	A	41 d	7	*e*	32
33	SC2066	1/16/2008	A	43 d	28	*k*	68
34	SC2068	1/16/2008	A	43 d	36	*e*	24
35	SC2073	1/16/2008	A	60 d	10	*e*	33

Table 2.9. Continued.

No.	ID	Received	Company	Age	PFGE type	*fla* type	PFGE and *fla* genotype
36	SC2211	5/21/2008	A	40 d	33	*e*	49
37	SC2213	5/21/2008	A	36 d	20	*k*	75
38	SC2235	5/21/2008	A	28 d	41	*a*	3
39	SC2215	5/21/2008	A	36 d	20	*k*	75
40	SC1870	6/28/2007	B	33 d	20	*f*	45
41	SC2094	1/17/2008	B	33 d	40	*e*	11
42	SC2288	5/22/2008	B	25 d	25	*e*	64
43	SC1436	12/13/2006	C	30 d	29	*l*	70
44	SC1444	12/13/2006	C	24 d	21	*e*	40
45	SC1445	12/13/2006	C	24 d	26	*f*	52
46	SC1454	12/13/2006	C	21 d	18	*f*	44
47	SC1458	12/13/2006	C	40 d	16	*e*	5
48	SC1460	12/13/2006	C	40 d	24	*f*	40
49	SC1472	12/13/2006	C	24 d	18	*f*	44
50	SC1617	3/7/2007	C	75 d	35	*e*	1
51	SC1634	3/7/2007	C	91 d	1	*f*	19
52	SC1789	6/27/2007	C	43 d	18	*f*	44
53	SC1790	6/27/2007	C	43 d	2	*f*	20
54	SC1799	6/27/2007	C	10 d	18	*f*	44
55	SC1799E-3	6/27/2007	C	10 d	18	*f*	44
56	SC2004	1/16/2008	C	59 d	39	*e*	16
57	SC2007	1/16/2008	C	56 d	12	*e*	6
58	SC2009	1/16/2008	C	37 d	38	*l*	65
59	SC2010	1/16/2008	C	37 d	38	*l*	65
60	SC2024	1/16/2008	C	12 d	12	*e*	6
61	SC2171	5/21/2008	C	25 d	18	*e*	42
62	SC2182	5/21/2008	C	17 d	38	*e*	17
63	SC2190	5/21/2008	C	29 d	33	*e*	49
64	SC2184	5/21/2008	C	35 d	8	*e*	30
65	SC1443	12/13/2006	C	24 d	37	*e*	25
66	SC1558	3/6/2007	D	25 d	21	*e*	41
67	SC1571	3/6/2007	D	14 d	29	*h*	61
68	SC1572	3/6/2007	D	14 d	29	*h*	61
69	SC1577	3/6/2007	D	25 d	29	*f*	58
70	SC1581E	3/6/2007	D	39 d	29	*a*	4

Table 2.9. Continued.

No.	ID	Received	Company	Age	PFGE type	*fla* type	PFGE and *fla* genotype
71	SC1583E	3/6/2007	D	39 d	29	*a*	4
72	SC1585	3/6/2007	D	17 d	29	*f*	58
73	SC1591	3/6/2007	D	29 d	8	*e*	30
74	SC1598	3/6/2007	D	21 d	41	*e*	11
75	SC1572E	3/6/2007	D	14 d	29	*h*	61
76	SC1583	3/6/2007	D	39 d	29	*a*	4
77	SC1599	3/6/2007	D	21 d	41	*e*	11
78	SC1727	6/26/2007	D	11 d	18	*f*	44
79	SC1730	6/26/2007	D	11 d	28	*f*	57
80	SC1733	6/26/2007	D	35 d	20	*f*	45
81	SC1737	6/26/2007	D	21 d	39	*f*	15
82	SC1742	6/26/2007	D	39 d	27	*f*	53
83	SC1743	6/26/2007	D	39 d	35	*d*	2
84	SC1745-3	6/26/2007	D	24 d	5	*f*	22
85	SC1747	6/26/2007	D	24 d	5	*f*	22
86	SC1756	6/26/2007	D	28 d	14	*e*	27
87	SC1761	6/26/2007	D	28 d	18	*k*	74
88	SC1765	6/26/2007	D	24 d	35	*a*	1
89	SC1770	6/26/2007	D	20 d	8	*e*	31
90	SC1775	6/26/2007	D	13 d	13	*a*	7
91	SC1779	6/26/2007	D	14 d	1	*e*	29
92	SC1783	6/26/2007	D	32 d	42	*f*	56
93	SC1786-3	6/26/2007	D	34 d	18	*f*	44
94	SC1745	6/26/2007	D	24 d	5	*f*	22
95	SC1788	6/26/2007	D	34 d	18	*f*	44
96	SC1943	1/15/2008	D	24 d	11	*a*	8
97	SC1948	1/15/2008	D	14 d	34	*f*	47
98	SC1954	1/15/2008	D	26 d	39	*f*	15
99	SC1958	1/15/2008	D	20 d	39	*e*	16
100	SC1961	1/15/2008	D	20 d	39	*e*	16
101	SC1969	1/15/2008	D	28 d	18	*f*	44
102	SC1970	1/15/2008	D	28 d	28	*f*	57
103	SC1978	1/15/2008	D	39 d	20	*f*	45
104	SC1994	1/15/2008	D	32 d	18	*f*	44
105	SC2111	5/20/2008	D	36 d	39	*e*	16

Table 2.9. Continued.

No.	ID	Received	Company	Age	PFGE type	*fla* type	PFGE and *fla* genotype
106	SC2114	5/20/2008	D	18 d	18	*f*	44
107	SC2135	5/20/2008	D	24 d	17	*a*	9
108	SC2153	5/20/2008	D	35 d	11	*a*	8
109	SC2162	5/20/2008	D	32 d	20	*f*	45
110	SC2163	5/20/2008	D	32 d	23	*g*	55
111	SC2565	6/16/2009	D	15 d	32	*e*	48
112	SC2578	6/16/2009	D	25 d	18	*k*	74
113	SC2583	6/16/2009	D	25 d	9	*e*	28
114	SC2595	6/16/2009	D	28 d	6	*f*	23
115	SC2605	6/16/2009	D	18 d	12	*i*	36
116	SC2623	6/17/2009	C	29 d	33	*e*	49
117	SC2633	6/17/2009	C	32 d	11	*i*	35
118	SC2640	6/17/2009	C	28 d	33	*e*	49
119	SC2642	6/17/2009	A	11 d	33	*f*	46
120	SC2648	6/17/2009	A	15 d	11	*i*	35
121	SC2651	6/17/2009	A	23 d	9	*e*	28
122	SC2656	6/17/2009	A	42 d	29	*f*	58
123	SC2659	6/17/2009	A	28 d	11	*i*	35
124	SC2661	6/17/2009	A	26 d	22	*f*	39
125	SC2671	6/17/2009	A	40 d	1	*k*	66
126	SC2675	6/17/2009	A	39 d	4	*f*	21
127	SC2680	6/17/2009	A	46 d	19	*e*	43
128	SC2682	6/24/2009	E	29 d	8	*e*	30
129	SC2688	6/24/2009	E	23 d	3	*e*	34
130	SC2722	6/25/2009	B	29 d	18	*e*	42
131	SC2726	6/25/2009	B	40 d	20	*j*	50
132	SC2732	6/25/2009	B	26 d	20	*j*	50
133	SC2753	6/25/2009	B	34 d	20	*j*	50
134	SC2758	6/25/2009	B	33 d	20	*j*	50
135	SC2767	6/25/2009	B	30 d	20	-	51
136	SC2780	12/1/2009	D	13 d	32	*k*	72
137	SC2800	12/1/2009	D	27 d	29	*e*	60
138	SC2818	12/1/2009	D	20 d	31	*k*	69
139	SC2835	12/1/2009	D	29 d	33	*k*	71
140	SC2843	12/2/2009	C	28 d	30	*f*	59
141	SC2849	12/2/2009	C	30 d	32	*e*	48

Table 2.9. Continued.

No.	ID	Received	Company	Age	PFGE type	*fla* type	PFGE and *fla* genotype
142	SC2893	12/2/2009	C	36 d	33	*f*	46
143	SC2895	12/2/2009	A	29 d	33	*f*	46
144	SC2909	12/2/2009	A	57 d	32	*e*	48
145	SC2916	12/2/2009	A	34 d	39	*f*	15
146	SC2923	12/2/2009	A	21 d	32	*e*	48
147	SC2924	12/2/2009	A	39 d	33	*e*	49
148	SC2937	12/2/2009	A	49 d	38	*f*	14
149	SC2944	12/2/2009	A	25 d	38	*f*	14
150	SC2984	12/3/2009	B	35 d	38	*f*	14
151	SC3018	12/9/2009	E	35 d	7	*i*	38
152	SC3021	12/9/2009	E	28 d	7	*e*	32

Table 2.10. The *fla* type for the 21 distinct PFGE types.

No.	PFGE type	Isolate	Date received [a]	Company [b]	*Fla* type
1	2	SC1790	6/27/2007	C	*f*
2	3	SC2688	6/24/2009	E	*e*
3	4	SC2675	6/17/2009	A	*f*
4	6	SC2595	6/16/2009	D	*f*
5	10	SC2073	1/16/2008	A	*e*
6	13	SC1775	6/26/2007	D	*a*
7	14	SC1756	6/26/2007	D	*e*
8	15	SC1649	3/7/2007	A	*g*
9	16	SC1458	12/13/2006	C	*e*
10	17	SC2135	5/20/2008	D	*a*
11	19	SC2680	6/17/2009	A	*e*
12	22	SC2661	6/17/2009	A	*f*
13	24	SC1460	12/13/2006	C	*f*
14	26	SC1445	12/13/2006	C	*f*
15	27	SC1742	6/26/2007	D	*f*
16	30	SC2843	12/2/2009	C	*f*
17	31	SC2818	12/1/2009	D	*k*
18	34	SC1948	1/15/2008	D	*f*
19	37	SC1443	12/13/2006	C	*e*
20	41	SC2235	5/21/2008	A	*a*
21	42	SC1783	6/26/2007	D	*f*

[a] There were 4 distinct PFGE type in 2006, 6 in 2007, 4 in 2008, and 8 in 2009.
[b] There were 6 distinct PFGE type from company A, none from company B, 6 from company C, 8 from company D, and one from company E.

Table 2.11. The result of MLST for 16 MDR *C. coli* isolates.

Isolate	*asp*A	*gln*A	*glt*A	*gly*A	*pgm*	*tkt*	*unc*A/ *atp*A	Sequence Type (ST)
SC1443	33	39	30	82	189	43	41	ST1101
SC1460	33	39	30	140	189	43	97	ST5131[a]
SC1585	33	39	30	82	189	43	41	ST1101
SC1617	33	39	30	82	189	43	41	ST1101
SC1680	115	39	30	140	104	43	41	ST1149
SC1742	33	39	30	140	104	47	97	ST1126
SC1779	33	39	30	82	113	47	41	ST889
SC1783	33	39	30	140	104	47	97	ST1126
SC1994	33	39	30	140	104	47	97	ST1126
SC2004	115	39	30	140	104	43	41	ST1149
SC2024	33	39	30	82	189	43	41	ST1101
SC2062	33	39	30	82	113	47	41	ST889
SC2073	33	39	30	82	189	43	41	ST1101
SC2135	33	39	30	82	189	43	41	ST1101
SC2211	33	39	30	82	189	43	41	ST1101
SC2818	33	39	30	82	189	43	41	ST1101

[a] ST5131 is the newly identified ST in this study.

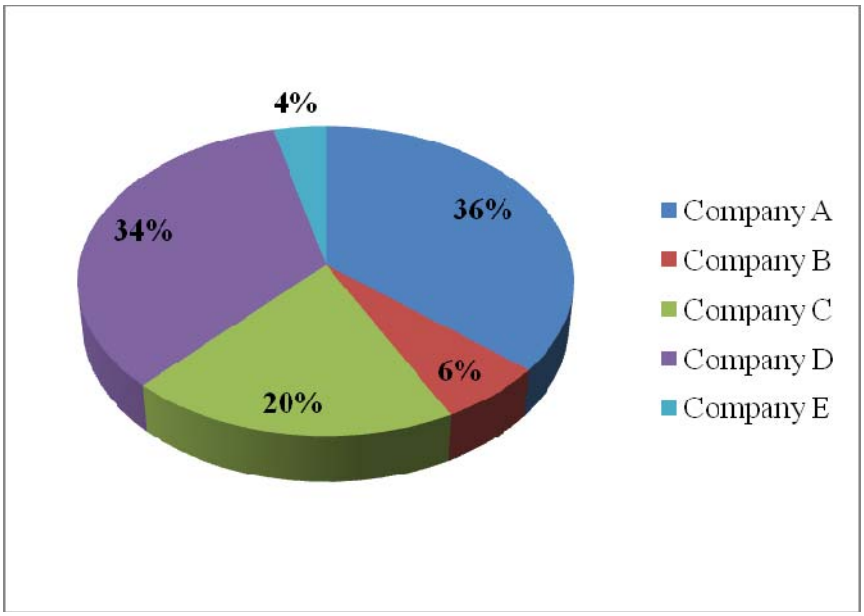


Figure 2.1. The distribution of the 152 MDR *C. coli* isolated from turkey grown by 5 different companies in this region.

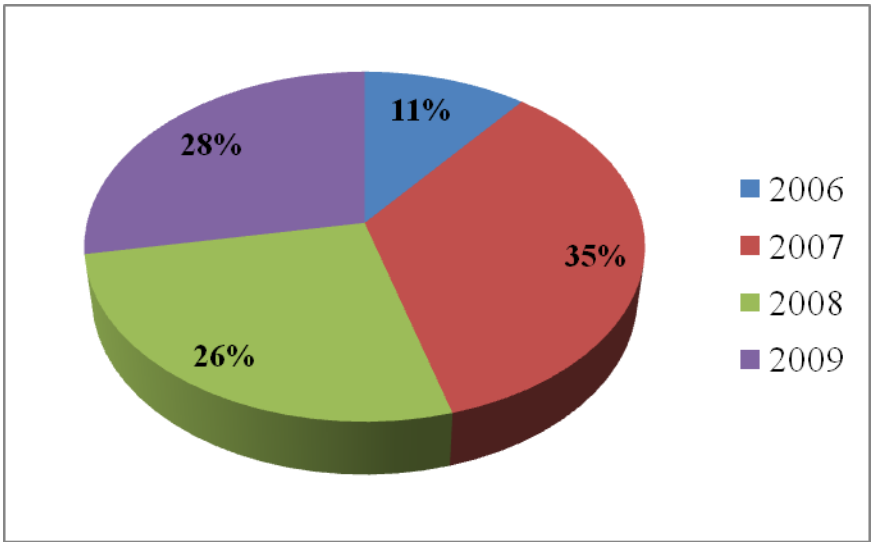


Figure 2,2. The distribution of 152 MDR *C. coli* isolates based on year isolated, from 2006 to 2009.

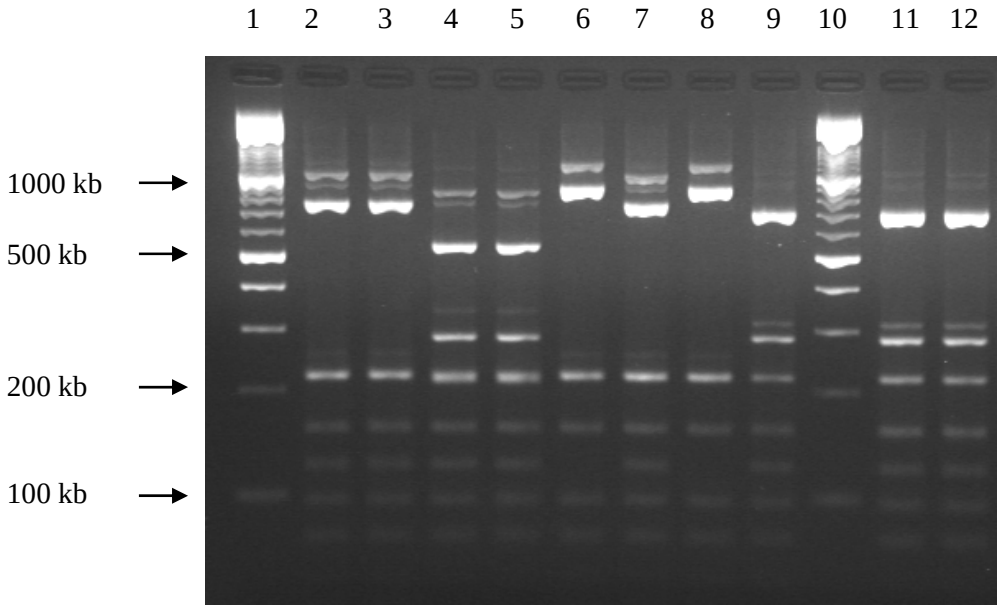


Figure 2.3. Representative of the gel electrophoresis of *fla* gene of MDR *C. coli* digested by restriction enzyme *Dde*I. The digested DNA was run on 3% agarose gel at 65V, for 150 minutes. Lane 1 and 10 are the DNA molecular weight XIV marker (100-1500 bp) (Roche Diagnostics GmbH, Germany); lane 2-4 and 6-9 are the MDR *C. coli* isolates.

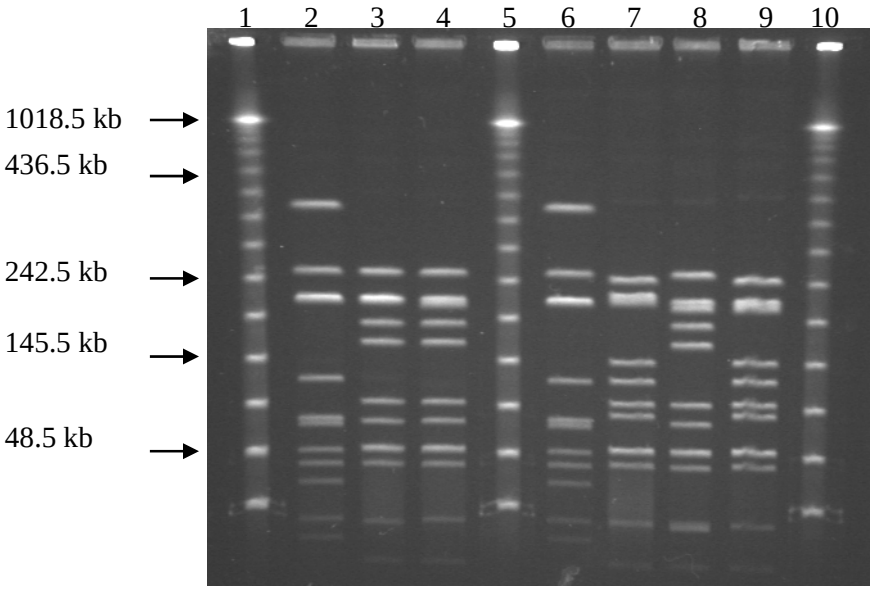
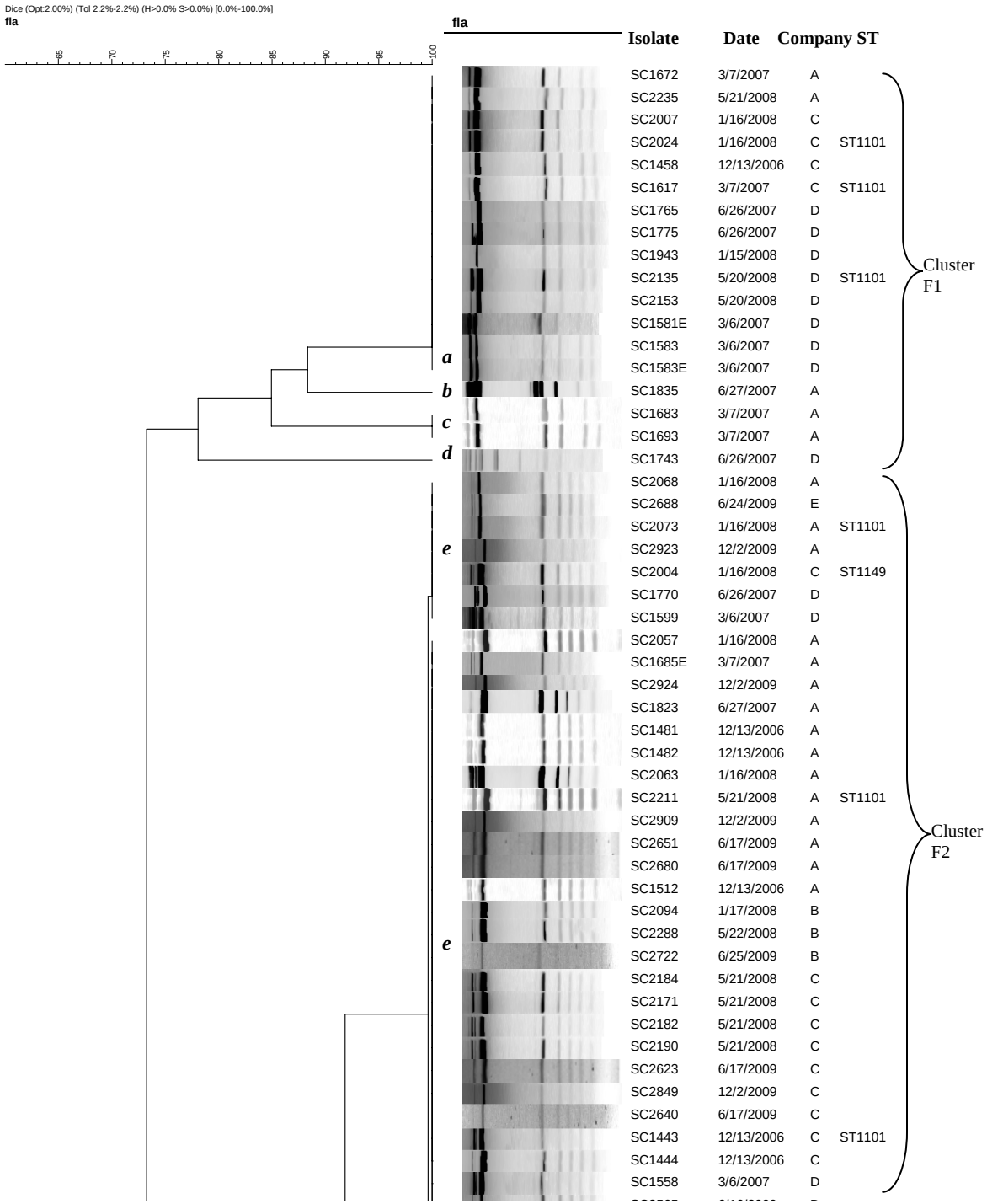
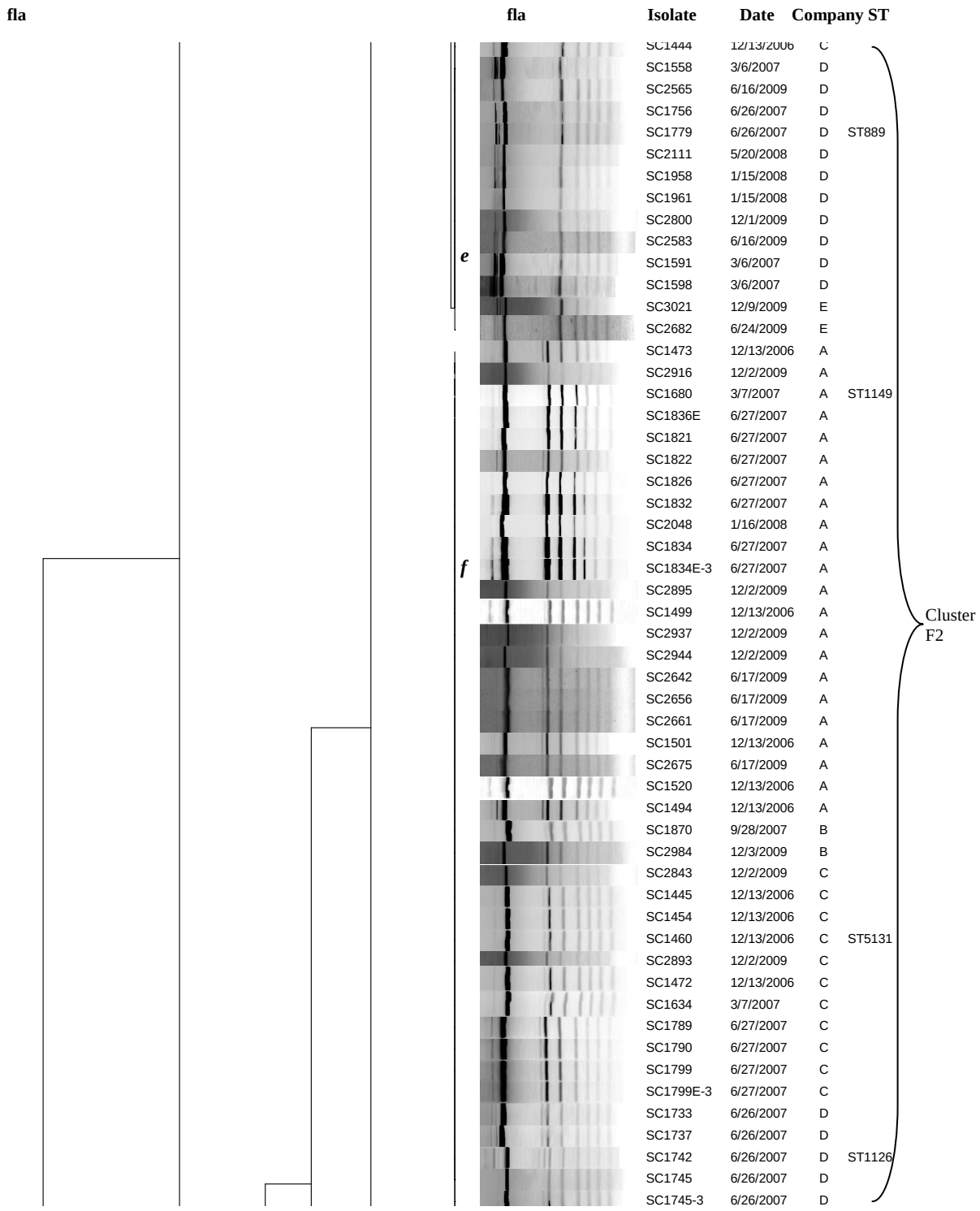


Figure 2.4. Representative pulsed-field gel electrophoresis of genomic DNA of MDR *C. coli* digested by restriction enzyme *Sma*I. The genomic DNA was run on 1% agarose gel at 6.75V, for 16 hr. Lane 1, 5 and 10 are the Lambda ladder pulsed-field gel electrophoresis (PFG) marker (Biolabs); lane 2-4 and 6-9 are MDR *C. coli* isolates.

Figure 2.5. Dendrogram generated by *fla* (*Dde*I) typing for 150 MDR *C. coli* isolates investigated in this study. Cluster analysis was performed by BioNumeric (version 4.6; Applied Maths).


Dice (Opt:2.00%) (Tol 2.2%-2.2%) (H>0.0% S>0.0%) [0.0%-100.0%]
fla
65
70
75
80
85
90
95
100
fla
Isolate Date Company ST
SC1672 3/7/2007 A
SC2235 5/21/2008 A
SC2007 1/16/2008 C
SC2024 1/16/2008 C ST1101
SC1458 12/13/2006 C
SC1617 3/7/2007 C ST1101
SC1765 6/26/2007 D
SC1775 6/26/2007 D
SC1943 1/15/2008 D
SC2135 5/20/2008 D ST1101
SC2153 5/20/2008 D
SC1581E 3/6/2007 D
SC1583 3/6/2007 D
SC1583E 3/6/2007 D
SC1835 6/27/2007 A
SC1683 3/7/2007 A
SC1693 3/7/2007 A
SC1743 6/26/2007 D
SC2068 1/16/2008 A
SC2688 6/24/2009 E
SC2073 1/16/2008 A ST1101
SC2923 12/2/2009 A
SC2004 1/16/2008 C ST1149
SC1770 6/26/2007 D
SC1599 3/6/2007 D
SC2057 1/16/2008 A
SC1685E 3/7/2007 A
SC2924 12/2/2009 A
SC1823 6/27/2007 A
SC1481 12/13/2006 A
SC1482 12/13/2006 A
SC2063 1/16/2008 A
SC2211 5/21/2008 A ST1101
SC2909 12/2/2009 A
SC2651 6/17/2009 A
SC2680 6/17/2009 A
SC1512 12/13/2006 A
SC2094 1/17/2008 B
SC2288 5/22/2008 B
SC2722 6/25/2009 B
SC2184 5/21/2008 C
SC2171 5/21/2008 C
SC2182 5/21/2008 C
SC2190 5/21/2008 C
SC2623 6/17/2009 C
SC2849 12/2/2009 C
SC2640 6/17/2009 C
SC1443 12/13/2006 C ST1101
SC1444 12/13/2006 C
SC1558 3/6/2007 D
a
b
c
d
e
e
Cluster F1
Cluster F2


fla
fla
Isolate
Date
Company
ST
SC1444 12/13/2006 C
SC1558 3/6/2007 D
SC2565 6/16/2009 D
SC1756 6/26/2007 D
SC1779 6/26/2007 D ST889
SC2111 5/20/2008 D
SC1958 1/15/2008 D
SC1961 1/15/2008 D
SC2800 12/1/2009 D
SC2583 6/16/2009 D
e
SC1591 3/6/2007 D
SC1598 3/6/2007 D
SC3021 12/9/2009 E
SC2682 6/24/2009 E
SC1473 12/13/2006 A
SC2916 12/2/2009 A
SC1680 3/7/2007 A ST1149
SC1836E 6/27/2007 A
SC1821 6/27/2007 A
SC1822 6/27/2007 A
SC1826 6/27/2007 A
SC1832 6/27/2007 A
SC2048 1/16/2008 A
SC1834 6/27/2007 A
f
SC1834E-3 6/27/2007 A
SC2895 12/2/2009 A
SC1499 12/13/2006 A
SC2937 12/2/2009 A
SC2944 12/2/2009 A
SC2642 6/17/2009 A
SC2656 6/17/2009 A
SC2661 6/17/2009 A
SC1501 12/13/2006 A
SC2675 6/17/2009 A
SC1520 12/13/2006 A
SC1494 12/13/2006 A
SC1870 9/28/2007 B
SC2984 12/3/2009 B
SC2843 12/2/2009 C
SC1445 12/13/2006 C
SC1454 12/13/2006 C
SC1460 12/13/2006 C ST5131
SC2893 12/2/2009 C
SC1472 12/13/2006 C
SC1634 3/7/2007 C
SC1789 6/27/2007 C
SC1790 6/27/2007 C
SC1799 6/27/2007 C
SC1799E-3 6/27/2007 C
SC1733 6/26/2007 D
SC1737 6/26/2007 D
SC1742 6/26/2007 D ST1126
SC1745 6/26/2007 D
SC1745-3 6/26/2007 D
Cluster F2

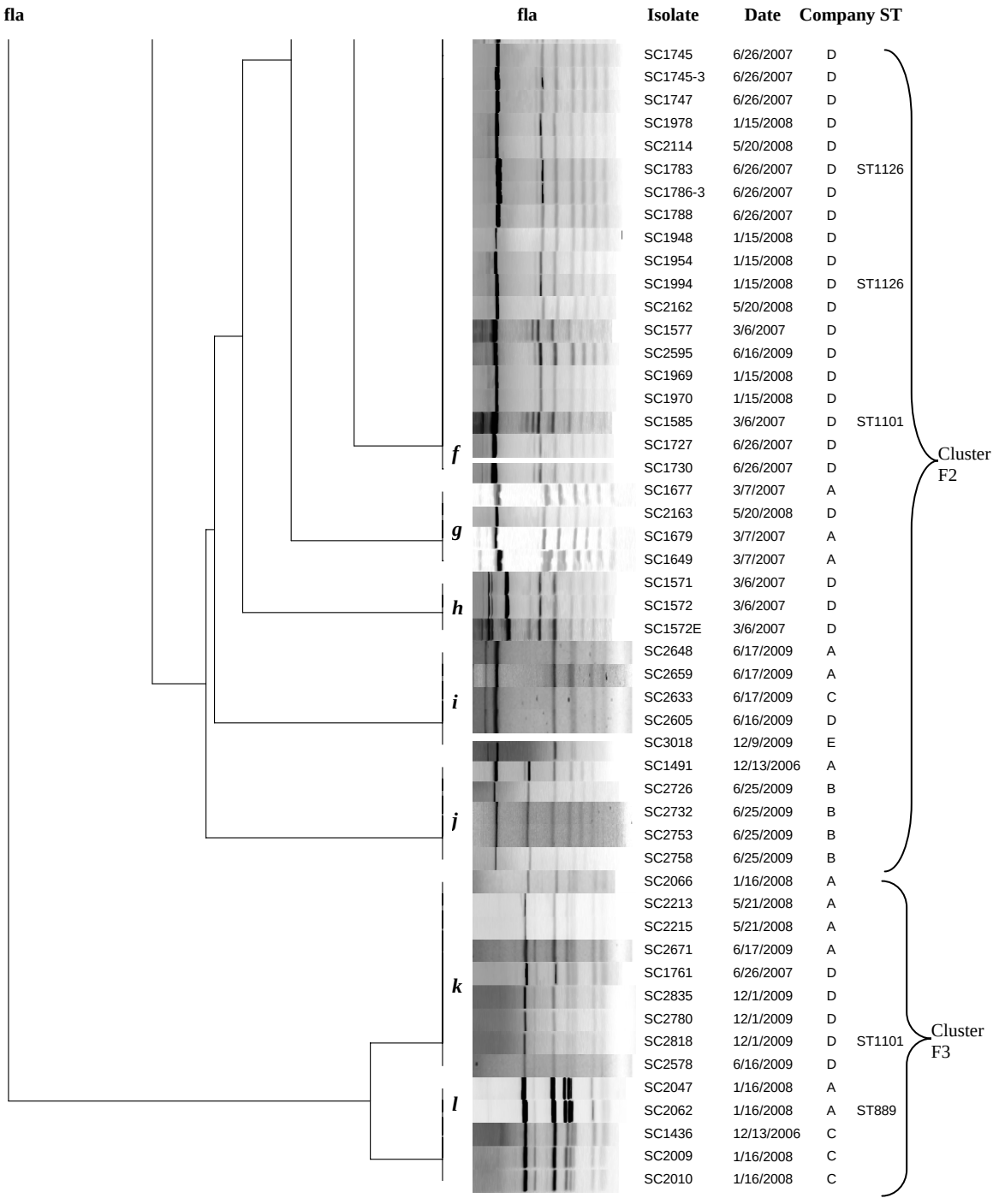
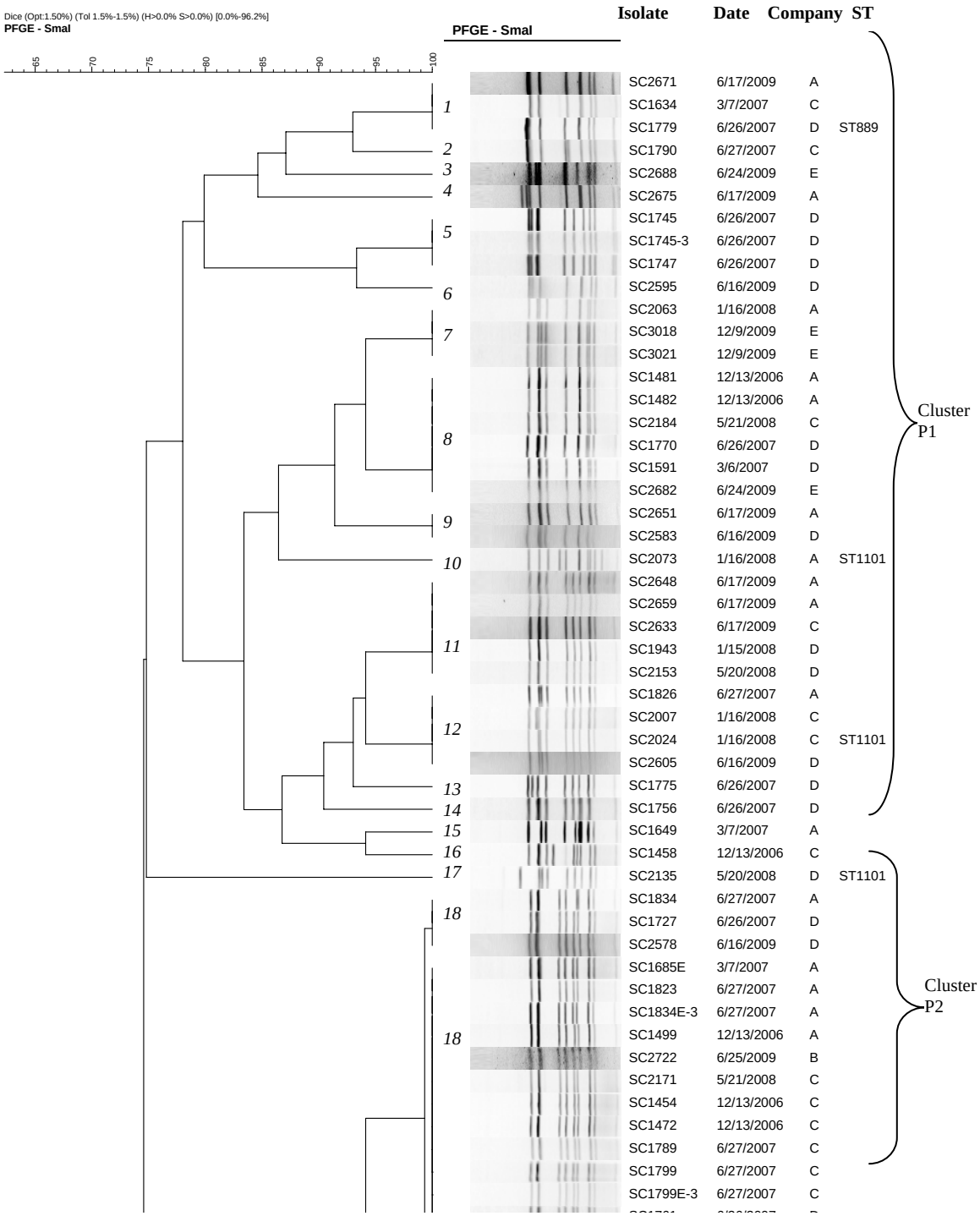
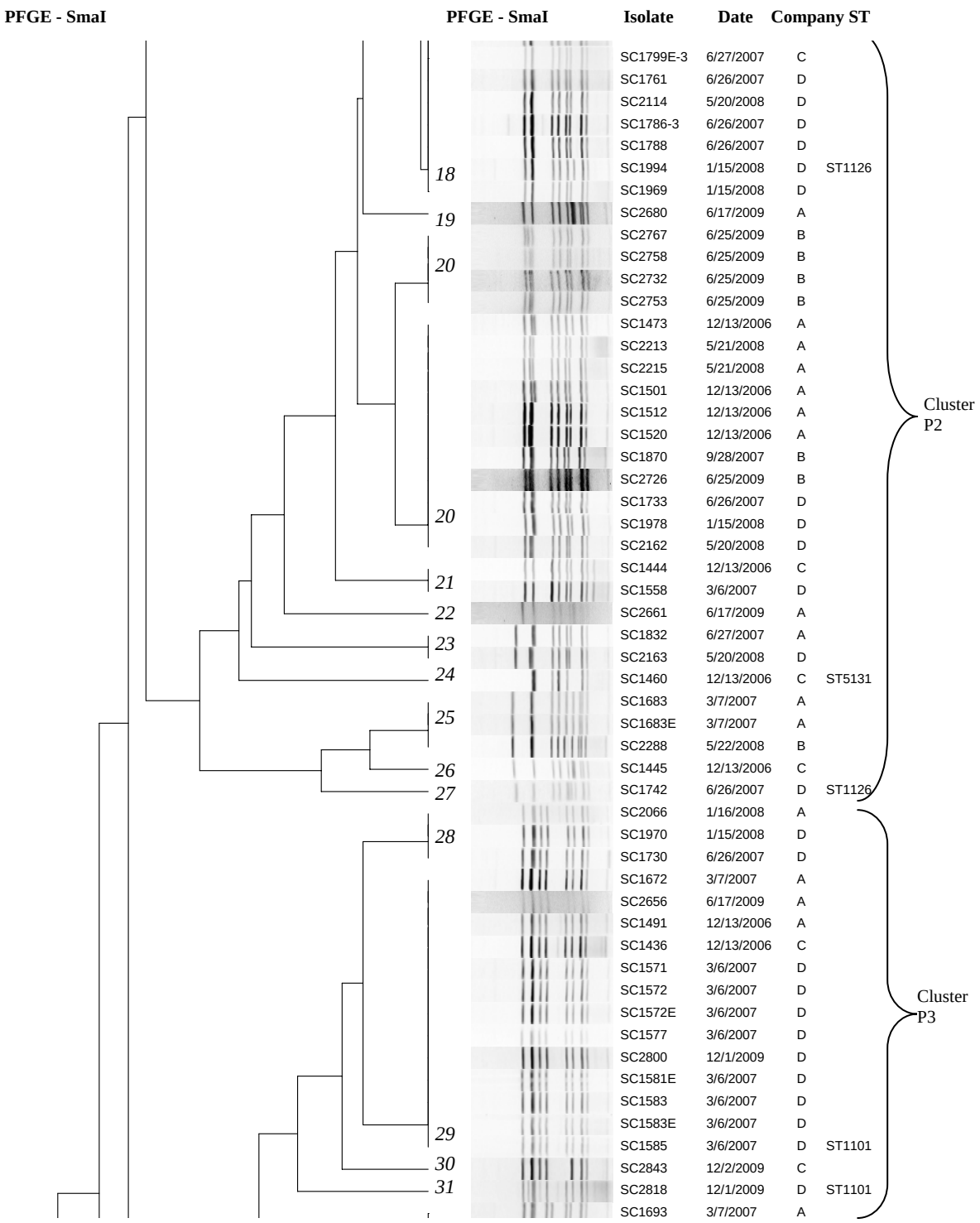

fla
fla
Isolate
Date
Company
ST
SC1745 6/26/2007 D
SC1745-3 6/26/2007 D
SC1747 6/26/2007 D
SC1978 1/15/2008 D
SC2114 5/20/2008 D
SC1783 6/26/2007 D ST1126
SC1786-3 6/26/2007 D
SC1788 6/26/2007 D
SC1948 1/15/2008 D
SC1954 1/15/2008 D
SC1994 1/15/2008 D ST1126
SC2162 5/20/2008 D
SC1577 3/6/2007 D
SC2595 6/16/2009 D
SC1969 1/15/2008 D
SC1970 1/15/2008 D
SC1585 3/6/2007 D ST1101
SC1727 6/26/2007 D
SC1730 6/26/2007 D
SC1677 3/7/2007 A
SC2163 5/20/2008 D
SC1679 3/7/2007 A
SC1649 3/7/2007 A
SC1571 3/6/2007 D
SC1572 3/6/2007 D
SC1572E 3/6/2007 D
SC2648 6/17/2009 A
SC2659 6/17/2009 A
SC2633 6/17/2009 C
SC2605 6/16/2009 D
SC3018 12/9/2009 E
SC1491 12/13/2006 A
SC2726 6/25/2009 B
SC2732 6/25/2009 B
SC2753 6/25/2009 B
SC2758 6/25/2009 B
SC2066 1/16/2008 A
SC2213 5/21/2008 A
SC2215 5/21/2008 A
SC2671 6/17/2009 A
SC1761 6/26/2007 D
SC2835 12/1/2009 D
SC2780 12/1/2009 D
SC2818 12/1/2009 D ST1101
SC2578 6/16/2009 D
SC2047 1/16/2008 A
SC2062 1/16/2008 A ST889
SC1436 12/13/2006 C
SC2009 1/16/2008 C
SC2010 1/16/2008 C
f
g
h
i
j
k
l
Cluster F2
Cluster F3

Figure 2.6. Dendrogram generated by PFGE (*Sma*I) profiles for 152 MDR *C. coli* isolates investigated in this study. Cluster analysis was performed by BioNumeric (version 4.6; Applied Maths).


Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-96.2%]
PFGE - SmaI
PFGE - SmaI
65
70
75
80
85
90
95
100
Isolate Date Company ST
SC2671 6/17/2009 A
SC1634 3/7/2007 C
SC1779 6/26/2007 D ST889
SC1790 6/27/2007 C
SC2688 6/24/2009 E
SC2675 6/17/2009 A
SC1745 6/26/2007 D
SC1745-3 6/26/2007 D
SC1747 6/26/2007 D
SC2595 6/16/2009 D
SC2063 1/16/2008 A
SC3018 12/9/2009 E
SC3021 12/9/2009 E
SC1481 12/13/2006 A
SC1482 12/13/2006 A
SC2184 5/21/2008 C
SC1770 6/26/2007 D
SC1591 3/6/2007 D
SC2682 6/24/2009 E
SC2651 6/17/2009 A
SC2583 6/16/2009 D
SC2073 1/16/2008 A ST1101
SC2648 6/17/2009 A
SC2659 6/17/2009 A
SC2633 6/17/2009 C
SC1943 1/15/2008 D
SC2153 5/20/2008 D
SC1826 6/27/2007 A
SC2007 1/16/2008 C
SC2024 1/16/2008 C ST1101
SC2605 6/16/2009 D
SC1775 6/26/2007 D
SC1756 6/26/2007 D
SC1649 3/7/2007 A
SC1458 12/13/2006 C
SC2135 5/20/2008 D ST1101
SC1834 6/27/2007 A
SC1727 6/26/2007 D
SC2578 6/16/2009 D
SC1685E 3/7/2007 A
SC1823 6/27/2007 A
SC1834E-3 6/27/2007 A
SC1499 12/13/2006 A
SC2722 6/25/2009 B
SC2171 5/21/2008 C
SC1454 12/13/2006 C
SC1472 12/13/2006 C
SC1789 6/27/2007 C
SC1799 6/27/2007 C
SC1799E-3 6/27/2007 C
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
18
Cluster P1
Cluster P2


PFGE - SmaI
PFGE - SmaI
Isolate
Date
Company
ST
SC1799E-3 6/27/2007 C
SC1761 6/26/2007 D
SC2114 5/20/2008 D
SC1786-3 6/26/2007 D
SC1788 6/26/2007 D
SC1994 1/15/2008 D ST1126
SC1969 1/15/2008 D
SC2680 6/17/2009 A
SC2767 6/25/2009 B
SC2758 6/25/2009 B
SC2732 6/25/2009 B
SC2753 6/25/2009 B
SC1473 12/13/2006 A
SC2213 5/21/2008 A
SC2215 5/21/2008 A
SC1501 12/13/2006 A
SC1512 12/13/2006 A
SC1520 12/13/2006 A
SC1870 9/28/2007 B
SC2726 6/25/2009 B
SC1733 6/26/2007 D
SC1978 1/15/2008 D
SC2162 5/20/2008 D
SC1444 12/13/2006 C
SC1558 3/6/2007 D
SC2661 6/17/2009 A
SC1832 6/27/2007 A
SC2163 5/20/2008 D
SC1460 12/13/2006 C ST5131
SC1683 3/7/2007 A
SC1683E 3/7/2007 A
SC2288 5/22/2008 B
SC1445 12/13/2006 C
SC1742 6/26/2007 D ST1126
SC2066 1/16/2008 A
SC1970 1/15/2008 D
SC1730 6/26/2007 D
SC1672 3/7/2007 A
SC2656 6/17/2009 A
SC1491 12/13/2006 A
SC1436 12/13/2006 C
SC1571 3/6/2007 D
SC1572 3/6/2007 D
SC1572E 3/6/2007 D
SC1577 3/6/2007 D
SC2800 12/1/2009 D
SC1581E 3/6/2007 D
SC1583 3/6/2007 D
SC1583E 3/6/2007 D
SC1585 3/6/2007 D ST1101
SC2843 12/2/2009 C
SC2818 12/1/2009 D ST1101
SC1693 3/7/2007 A
18
19
20
20
21
22
23
24
25
26
27
28
29
30
31
Cluster P2
Cluster P3

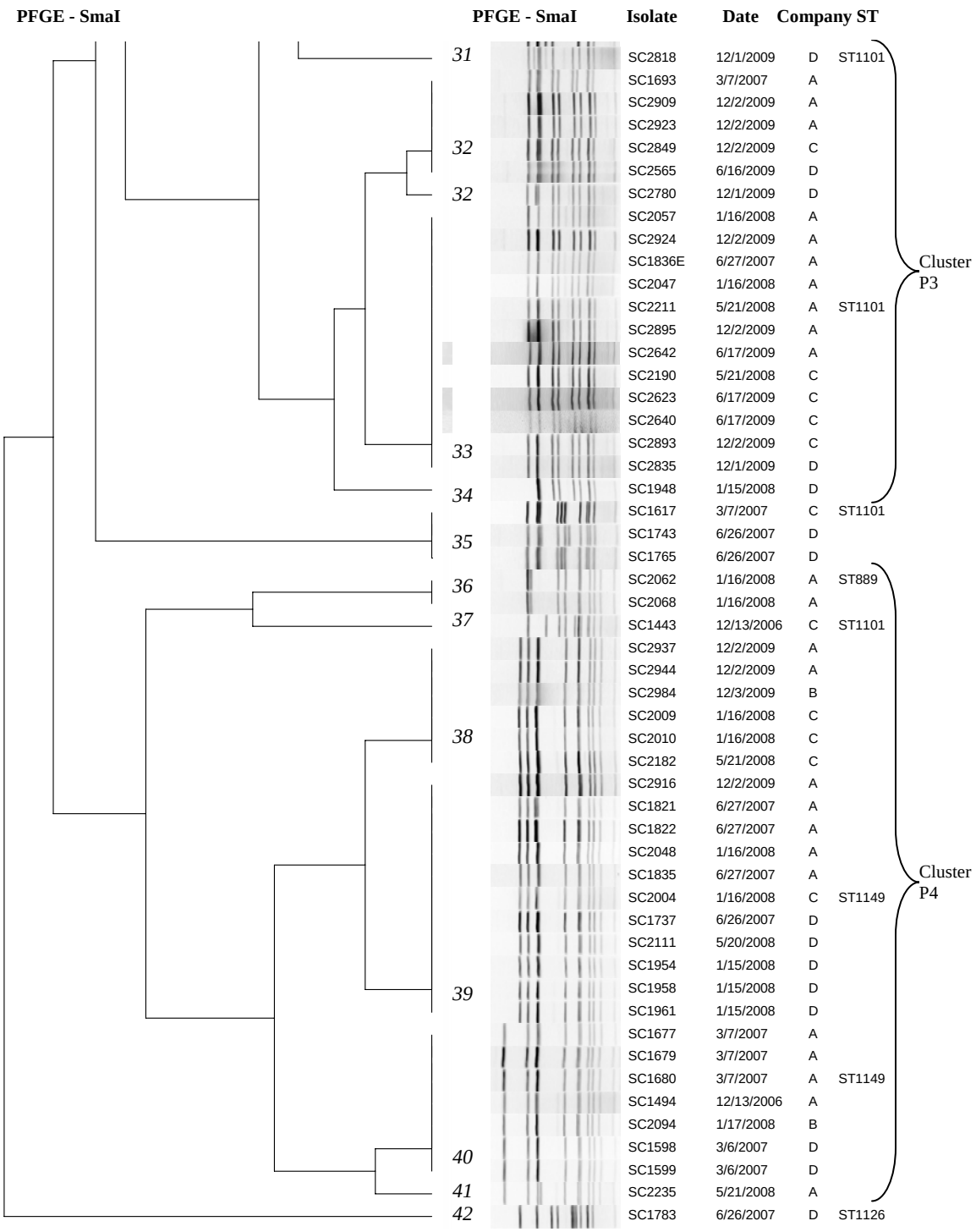
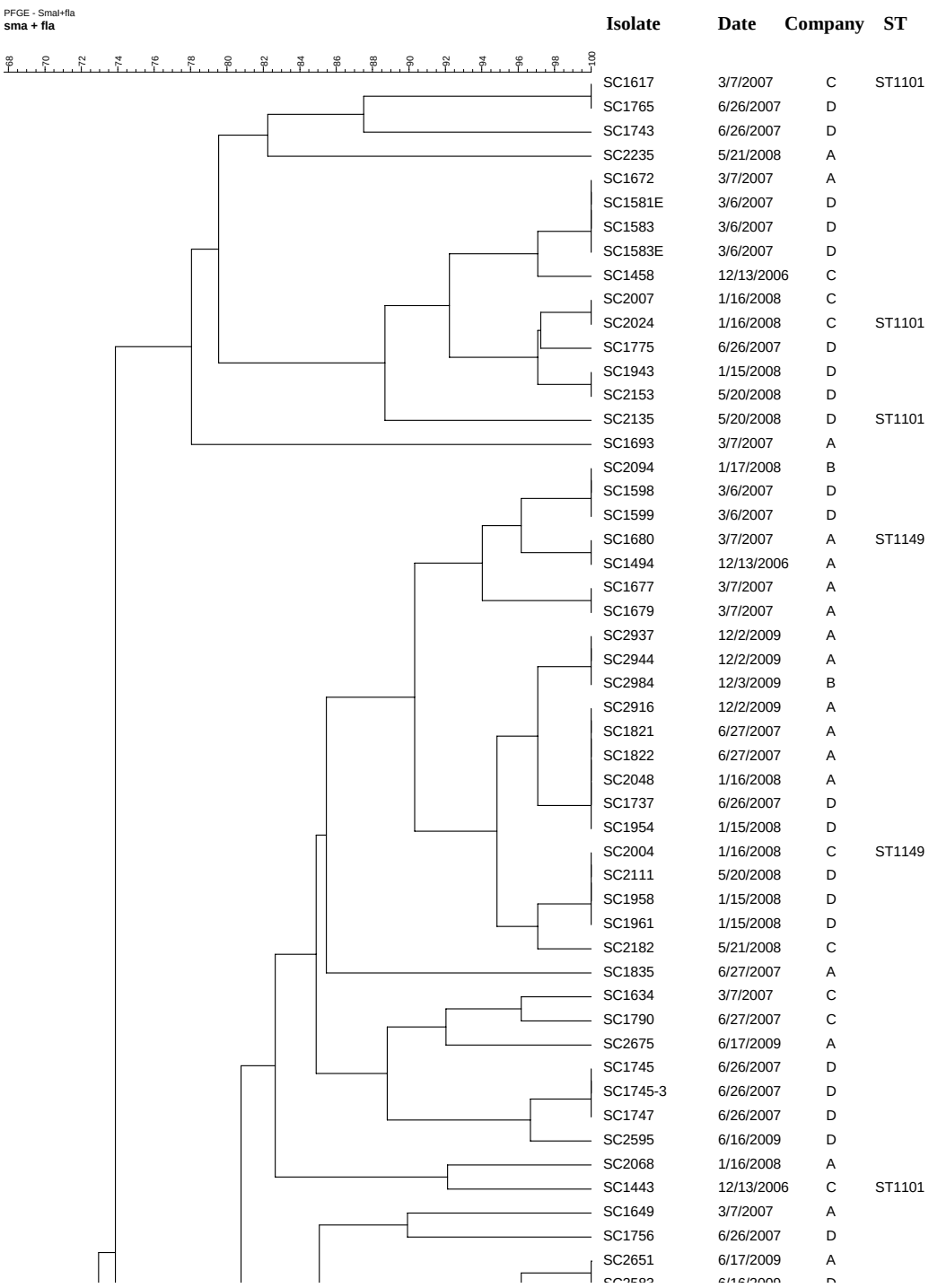

PFGE - SmaI
PFGE - SmaI
Isolate
Date
Company
ST
31
32
32
33
34
35
36
37
38
39
40
41
42
SC2818 12/1/2009 D ST1101
SC1693 3/7/2007 A
SC2909 12/2/2009 A
SC2923 12/2/2009 A
SC2849 12/2/2009 C
SC2565 6/16/2009 D
SC2780 12/1/2009 D
SC2057 1/16/2008 A
SC2924 12/2/2009 A
SC1836E 6/27/2007 A
SC2047 1/16/2008 A
SC2211 5/21/2008 A ST1101
SC2895 12/2/2009 A
SC2642 6/17/2009 A
SC2190 5/21/2008 C
SC2623 6/17/2009 C
SC2640 6/17/2009 C
SC2893 12/2/2009 C
SC2835 12/1/2009 D
SC1948 1/15/2008 D
SC1617 3/7/2007 C ST1101
SC1743 6/26/2007 D
SC1765 6/26/2007 D
SC2062 1/16/2008 A ST889
SC2068 1/16/2008 A
SC1443 12/13/2006 C ST1101
SC2937 12/2/2009 A
SC2944 12/2/2009 A
SC2984 12/3/2009 B
SC2009 1/16/2008 C
SC2010 1/16/2008 C
SC2182 5/21/2008 C
SC2916 12/2/2009 A
SC1821 6/27/2007 A
SC1822 6/27/2007 A
SC2048 1/16/2008 A
SC1835 6/27/2007 A
SC2004 1/16/2008 C ST1149
SC1737 6/26/2007 D
SC2111 5/20/2008 D
SC1954 1/15/2008 D
SC1958 1/15/2008 D
SC1961 1/15/2008 D
SC1677 3/7/2007 A
SC1679 3/7/2007 A
SC1680 3/7/2007 A ST1149
SC1494 12/13/2006 A
SC2094 1/17/2008 B
SC1598 3/6/2007 D
SC1599 3/6/2007 D
SC2235 5/21/2008 A
SC1783 6/26/2007 D ST1126
Cluster P3
Cluster P4

Figure 2.7. Dendrogram generated from *fla* (*Dde*I) and PFGE (*Sma*I) profiles of the 152 MDR *C. coli* used in this study.



Isolate	Date	Company	ST
SC1617	3/7/2007	C	ST1101
SC1765	6/26/2007	D	
SC1743	6/26/2007	D	
SC2235	5/21/2008	A	
SC1672	3/7/2007	A	
SC1581E	3/6/2007	D	
SC1583	3/6/2007	D	
SC1583E	3/6/2007	D	
SC1458	12/13/2006	C	
SC2007	1/16/2008	C	
SC2024	1/16/2008	C	ST1101
SC1775	6/26/2007	D	
SC1943	1/15/2008	D	
SC2153	5/20/2008	D	
SC2135	5/20/2008	D	ST1101
SC1693	3/7/2007	A	
SC2094	1/17/2008	B	
SC1598	3/6/2007	D	
SC1599	3/6/2007	D	
SC1680	3/7/2007	A	ST1149
SC1494	12/13/2006	A	
SC1677	3/7/2007	A	
SC1679	3/7/2007	A	
SC2937	12/2/2009	A	
SC2944	12/2/2009	A	
SC2984	12/3/2009	B	
SC2916	12/2/2009	A	
SC1821	6/27/2007	A	
SC1822	6/27/2007	A	
SC2048	1/16/2008	A	
SC1737	6/26/2007	D	
SC1954	1/15/2008	D	
SC2004	1/16/2008	C	ST1149
SC2111	5/20/2008	D	
SC1958	1/15/2008	D	
SC1961	1/15/2008	D	
SC2182	5/21/2008	C	
SC1835	6/27/2007	A	
SC1634	3/7/2007	C	
SC1790	6/27/2007	C	
SC2675	6/17/2009	A	
SC1745	6/26/2007	D	
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SC2595	6/16/2009	D	
SC2068	1/16/2008	A	
SC1443	12/13/2006	C	ST1101
SC1649	3/7/2007	A	
SC1756	6/26/2007	D	
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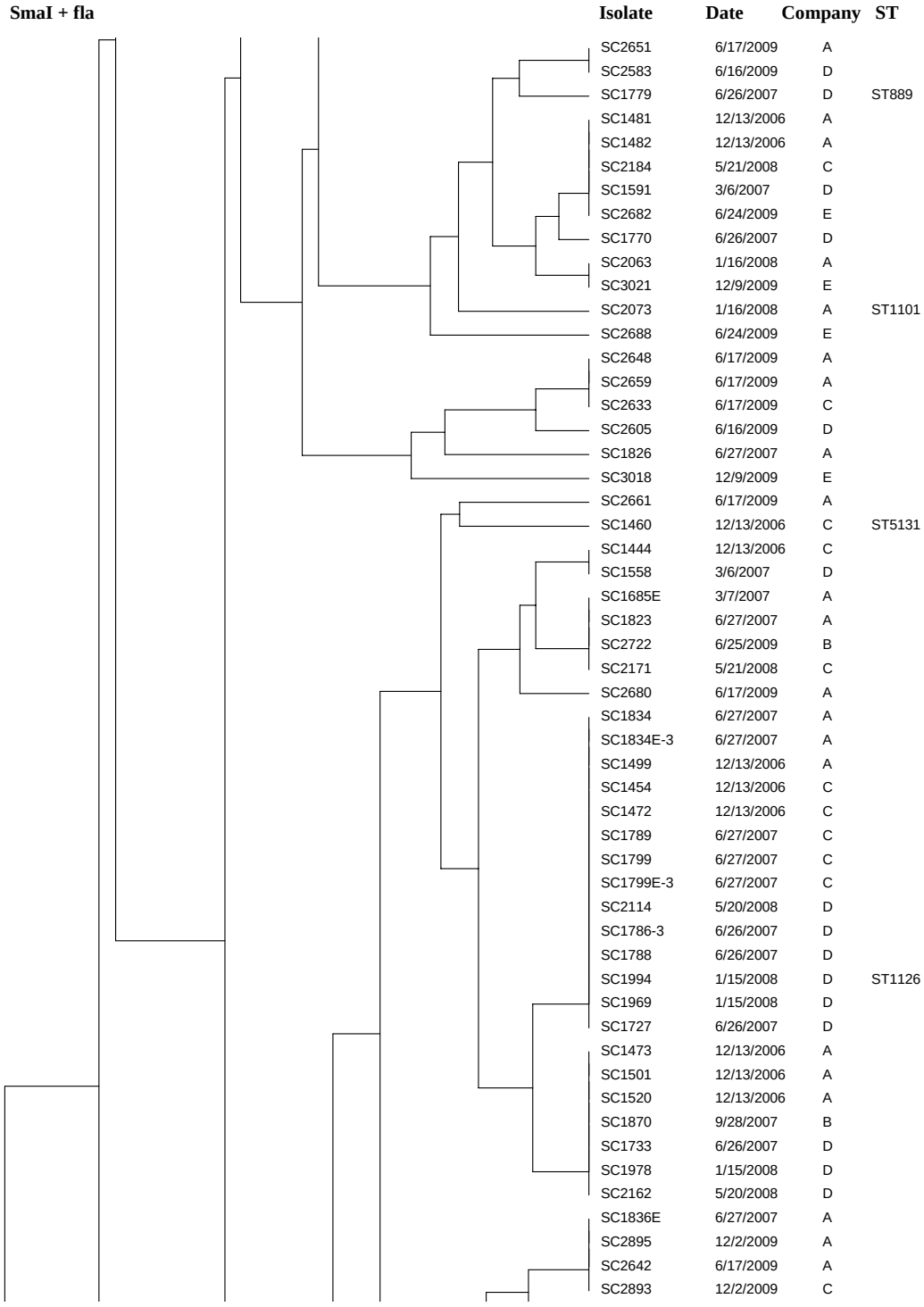
SmaI + fla
Isolate
Date
Company
ST
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SC1482 12/13/2006 A
SC2184 5/21/2008 C
SC1591 3/6/2007 D
SC2682 6/24/2009 E
SC1770 6/26/2007 D
SC2063 1/16/2008 A
SC3021 12/9/2009 E
SC2073 1/16/2008 A ST1101
SC2688 6/24/2009 E
SC2648 6/17/2009 A
SC2659 6/17/2009 A
SC2633 6/17/2009 C
SC2605 6/16/2009 D
SC1826 6/27/2007 A
SC3018 12/9/2009 E
SC2661 6/17/2009 A
SC1460 12/13/2006 C ST5131
SC1444 12/13/2006 C
SC1558 3/6/2007 D
SC1685E 3/7/2007 A
SC1823 6/27/2007 A
SC2722 6/25/2009 B
SC2171 5/21/2008 C
SC2680 6/17/2009 A
SC1834 6/27/2007 A
SC1834E-3 6/27/2007 A
SC1499 12/13/2006 A
SC1454 12/13/2006 C
SC1472 12/13/2006 C
SC1789 6/27/2007 C
SC1799 6/27/2007 C
SC1799E-3 6/27/2007 C
SC2114 5/20/2008 D
SC1786-3 6/26/2007 D
SC1788 6/26/2007 D
SC1994 1/15/2008 D ST1126
SC1969 1/15/2008 D
SC1727 6/26/2007 D
SC1473 12/13/2006 A
SC1501 12/13/2006 A
SC1520 12/13/2006 A
SC1870 9/28/2007 B
SC1733 6/26/2007 D
SC1978 1/15/2008 D
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SC1836E 6/27/2007 A
SC2895 12/2/2009 A
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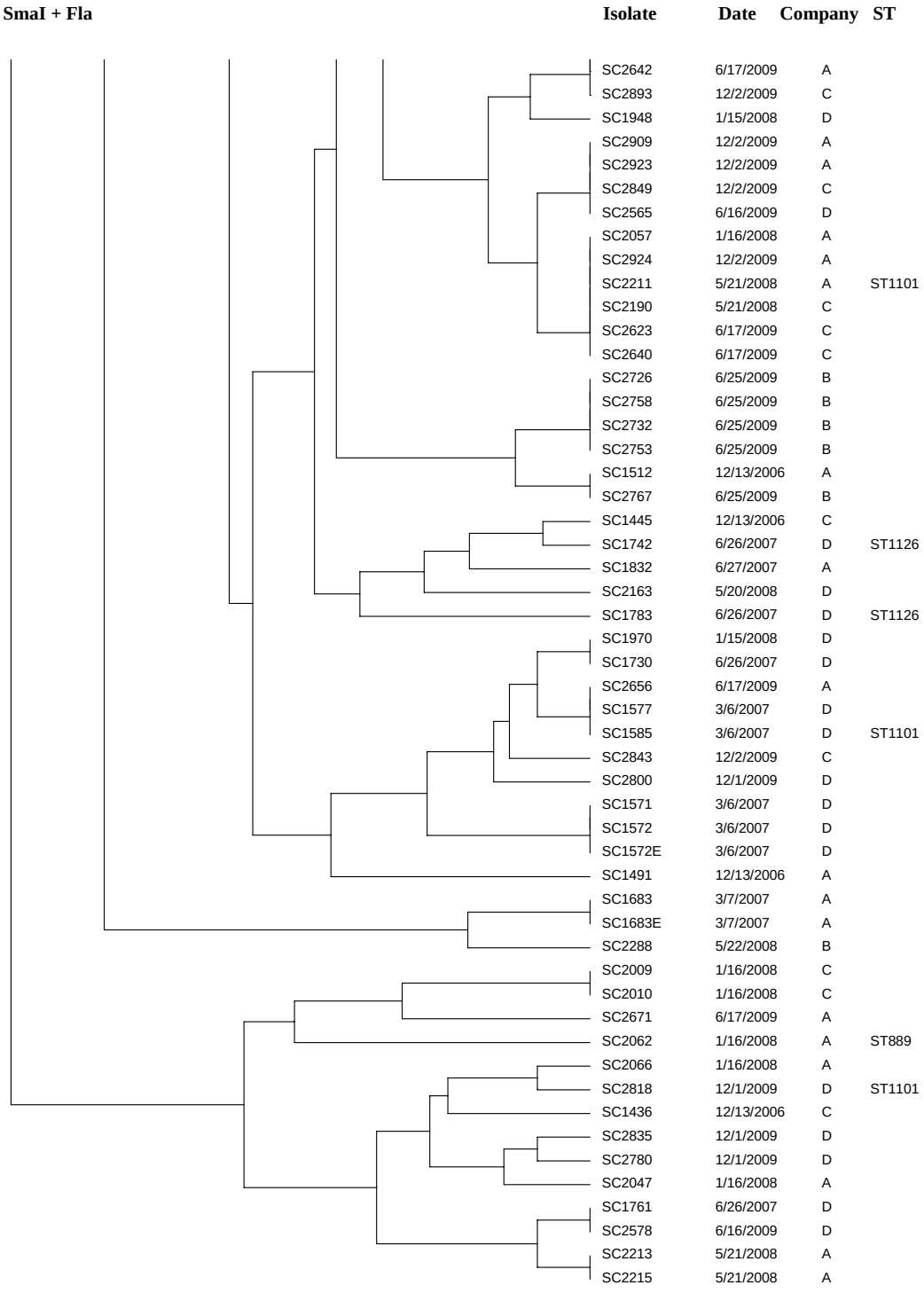
SC2893 12/2/2009 C


SmaI + Fla
Isolate
Date
Company
ST
SC2642 6/17/2009 A
SC2893 12/2/2009 C
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SC2909 12/2/2009 A
SC2923 12/2/2009 A
SC2849 12/2/2009 C
SC2565 6/16/2009 D
SC2057 1/16/2008 A
SC2924 12/2/2009 A
SC2211 5/21/2008 A ST1101
SC2190 5/21/2008 C
SC2623 6/17/2009 C
SC2640 6/17/2009 C
SC2726 6/25/2009 B
SC2758 6/25/2009 B
SC2732 6/25/2009 B
SC2753 6/25/2009 B
SC1512 12/13/2006 A
SC2767 6/25/2009 B
SC1445 12/13/2006 C
SC1742 6/26/2007 D ST1126
SC1832 6/27/2007 A
SC2163 5/20/2008 D
SC1783 6/26/2007 D ST1126
SC1970 1/15/2008 D
SC1730 6/26/2007 D
SC2656 6/17/2009 A
SC1577 3/6/2007 D
SC1585 3/6/2007 D ST1101
SC2843 12/2/2009 C
SC2800 12/1/2009 D
SC1571 3/6/2007 D
SC1572 3/6/2007 D
SC1572E 3/6/2007 D
SC1491 12/13/2006 A
SC1683 3/7/2007 A
SC1683E 3/7/2007 A
SC2288 5/22/2008 B
SC2009 1/16/2008 C
SC2010 1/16/2008 C
SC2671 6/17/2009 A
SC2062 1/16/2008 A ST889
SC2066 1/16/2008 A
SC2818 12/1/2009 D ST1101
SC1436 12/13/2006 C
SC2835 12/1/2009 D
SC2780 12/1/2009 D
SC2047 1/16/2008 A
SC1761 6/26/2007 D
SC2578 6/16/2009 D
SC2213 5/21/2008 A
SC2215 5/21/2008 A

CHAPTER 3

The presence of *CJE1461* gene in *C. coli* isolates from turkey flocks.

3.1. **ABSTRACT**

Campylobacter coli are naturally competent for DNA uptake, and this can increase genetic diversity leading to altered bacterial characteristics. In the present study, a total of 214 MDR and non-MDR *C. coli* isolates from turkey flocks were analyzed with *hip* primers that are commonly used for *C. jejuni* identification. Two MDR *C. coli* strains (SC2004 and SC1585) produced PCR products of approximately 380 bp with PCR using *hip* primers. Sequence analysis revealed that these PCR products matched with the DNA of *CJE1461* gene from *C. jejuni* RM1221, belonging to a putative prophage. Further analysis using primers derived from *CJE1461* confirmed the presence of this gene in SC2004 and SC1585 as well in several other isolates. Overall, 113/178 (63%) of the MDR *C. coli* and 3/9 (33.3%) of the non-MDR *C. coli* were identified as prophage-positive based on PCR using *hip* primers. The number of prophage-positive MDR *C. coli* isolates increased from 27% in 2002-2004 isolates to 81% in isolates from 2009. PFGE and *fla* analysis showed that the majority (98%) of the prophage-positive MDR *C. coli* had genotypes different from those of the prophage-negative isolates. MLST data revealed ST1101, ST1149 and ST1126 among the prophage-positive MDR *C. coli* isolates while the prophage-negative MDR *C. coli* isolates mostly belonged to one cluster that included v isolates of ST1126. Findings from this study clearly indicate that the *CJE1461* gene was present in a majority of our MDR *C. coli* isolates. Further studies are needed to assess the role of this gene in increasing the genetic diversity and possibly the fitness of MDR *C. coli* that colonize turkeys.

3.2. INTRODUCTION

Polymerase Chain Reaction (PCR) tests have been developed to differentiate between the closely related species *Campylobacter coli* and *C. jejuni*. The *ceu*E primers amplify 978 bp DNA from *ceu*E, a gene that is present in both *C. coli* and *C. jejuni* but exhibits sequence differences between these two species. *Due to these sequence differences,* the selected *ceu*E primers only amplified DNA from *C. coli* (Houng *et al*., 2001). The *hip* primers was developed by Marshall *et al*. (1999) based on the *hippuricase* gene sequence reported by Hani and Chan (1995). The *hippuricase* gene is present only in *C. jejuni* and encodes hippuricase, an enzyme that hydrolyzes hippurate to benzoic acid and glycine (Hani and Chan, 1995).

One of the major contributors to the genetic diversity in bacterial species is horizontal gene transfer (HGT) (Dutta and Pan, 2002). *C. coli* and *C. jejuni* are known to be naturally competent for uptake of DNA from the environment (Wang and Taylor, 1990; Gaasbeek *et al*., 2010). The new DNA fragments can be integrated into the bacterial genome and can change bacterial characteristics (Chen and Dubneu, 2004). This ability can increase genetic diversity in *C. coli* and *C. jejuni* (Wassenaar and Newell, 2000). Studies from our laboratory have demonstrated the possibility of interspecies transfer of chromosomal DNA between *C. coli* and *C. jejuni* (Chan *et al*., 2008).

Genome sequencing of *C. jejuni* RM1221 identified four integrated elements, CJIE1-CJIE4 (Parker *et al*., 2006). CJIE1 was previously known as the *Campylobacter* Mu-like phage (CMLPI); it was found to encode proteins that were similar to bacteriophage Mu and

other Mu-like proteins, suggesting that this genomic element might have come from a bacteriophage (Parker *et al*., 2006).

CJIE2 and CJIE4 were also predicted to have been derived from putative phage DNA (Parker *et al*., 2006; Fouts *et al*., 2005). Although no known virulence determinants have been found in any of the integrated elements, recent studies have demonstrated that CJIE2 and CJIE4 encoded nonspecific endonucleases that inhibit the natural transformation ability of *C. jeuni* strains (Gaasbeek *et al*., 2010). Only CJIE3 was suggested to have originated from a genomic island or integrated plasmid (Fouts *et al*., 2005; Parker *et al*., 2006). At least one study had demonstrated the presence of *CJIE1* or *CJIE3* genes in some *C. coli* (Parker *et al*., 2006). However, *CJIE2* and *CJIE4* have not been detected in *C. coli*.

Our laboratory has characterized multidrug resistant (MDR) *C. coli* strains from turkeys. In the course of these studies we investigated the possibility that certain strains harbor a gene related to sequences in the integrated element CJIE4, that was previously found in *C. jejuni* strains.

3.3. MATERIALS AND METHODS

***Campylobacter* strains and growth condition.** The *Campylobacter coli* isolates in this study were from the *Campylobacter* culture collection in our laboratory and had been isolated between 2002 and 2009 from commercially grown turkeys in North Carolina and South Carolina. The majority of the isolates (n=178) were defined as multidrug resistant (MDR) *C. coli* based on resistance to six types of antimicrobial agents (ciprofloxacin, erythromycin, kanamycin, nalidixic acid, streptomycin, and tetracycline). The bacteria were

isolated from cecal contents of turkeys using direct plating as described previously (Smith *et al.*, 2004). Bacteria were grown on tryptic soy agar with 5% sheep blood (Remel, Lenexa, KS) at 42°C for 48 hours under microaerobic conditions as described previously (Smith *et al.*, 2004). Bacteria were preserved at -80°C in cryovials containing 1.0 mL of brain heart infusion broth (Difco, Sparks, MD) with 20% glycerol.

DNA extraction and bacterial subtyping using *fla* and PFGE were conducted as described in Chapter 2.

PCR amplification. Genomic DNA was extracted using Qiagen DNeasy kit (Qiagen, Valencia, CA) as described previously (Smith *et al.*, 2004). PCR was used to differentiate between *Campylobacter* species using species-specific primers, *hip* primers for *C. jejuni* and *ceuE* primers for *C. coli,* as described (Smith *et al.*, 2004). The primer set *CJE1461* F (5'-CGC ACC ACC TGT TCC TGC GT-3') and *CJE1461* R (5'-ACG GTG CTT CTT TGG CGG GT-3') was used to determine the presence of the *CJE1461* gene in *C. coli* isolates. This primer was previously designed in our lab (unpublished data) based on the *CJE1461* gene in *C. jejuni* RM1221 (GenBank accession number NC 003912). The expected size generated by this primer is 230 bp (Figure 3.1). PCR reactions were carried out as described (Smith *et al*., 2004).

DNA sequencing of the PCR products. The primers used to amplify the PCR products were also used for sequencing of the product. PCR amplicons were identified by gel electrophoresis on 1% agarose gel and purified by QIAquick gel extraction kit (Qiagen, Valencia, CA). Amplicons were sequenced at the Genomic Research Laboratory of North Carolina State University. Sequence data were analyzed and assembled using the online

application ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/). BLASTN on the NCBI website (http://www.ncbi.nlm.gov.BLAST/) was used to search for homology in the DNA sequence database.

3.4. RESULTS

A total of 214 *C. coli* isolates from five different companies were analyzed in this study. The majority of the isolates (n=182) were MDR isolates isolated from 2002 to 2009 (Table 3.1), and the remaining were *C. coli* isolates sensitive to at least one type of antimicrobial agent in the panel. We only analyzed the non-MDR isolates that we received between 2009 and 2010 (Table 3.2).

The *ceuE* primers that were used to identify *C. coli* should amplify DNA from *ceu*E, while the *hip* primers should amplify DNA from the hippuricase gene that is only present in *C. jejuni*. The size of amplicon with the *ceu*E primers is 892 base pairs (bp) and with the *hip* primers it is 176 bp (Marshall *et al*., 1999; Houng *et al*., 2001). PCR reactions using mixed primers that contained these two primer pairs should be able to distinguish between *C. coli* and *C. jejuni*. However, in the course of our study we frequently observed the presence of a third type of DNA fragment with size approximately 380 bp (Figure 3.2), which later in this presentation was identified as the prophage band. Further analysis using each primer pair separately revealed that this 380 bp band was only obtained with the *hip* primers.

We sequenced the 380 bp DNA fragments that were amplified using *hip* primers from two isolates (SC2004 and SC1585). We found that the DNA sequences were identical to each other and to those previously obtained in our laboratory from three *C. coli* isolates using the *hip* primers (R. M. Siletzky, unpublished data) (Figure 3.5). BLASTN was used to

search for homologous sequences in the GenBank database. All the DNA sequences amplified by *hip* primers matched with *CJE1461* (nucleotides 3956 to 4271), from *C. jejuni* RM1221 (accession number NC 0023912).

The CJE1461 primers used in this study were designed specifically to amplify the DNA within the *CJE1461* gene. Any positive PCR amplification in our *C. coli* isolates should indicate the presence of this *CJE1461* gene. DNA sequences amplified by *CJE1461* primers were matched with nucleotides number 2267 to 2400 (Figure 3.6 and Figure 3.7), respectively.

Twenty six MDR *C. coli* isolated between 2002 and 2004 were analyzed with both *hip* and *CJE1461* primers. All 152 MDR *C. coli* isolates from 2005 to 2009 were also analyzed with the *hip* primers but only 24 were analyzed with *CJE1461* primers. *CJE1461* primers were used to analyzed the 2009 and 2010 *C. coli* isolates (n=36) that were sensitive to at least to one antimicrobial agent, and only 9 isolates were tested with the *hip* primers. The results from analysis with *hip* and *CJE1461* primers on each *C. coli* strain used in this study are presented in Table 3.1 and Table 3.2.

Overall, 113/178 (63%) of the MDR *C. coli* isolates were prophage-positive, and the percentage of prophage-positive in MDR *C. coli* gradually increased from 27% in 2002-2004 to 81% in 2009 (Table 3.3). The prophage band was identified in isolates from all five companies with isolates from company A, C and D being 74%, 70% and 60% prophage-positive, respectively. Companies B and E only have 35% and 50% prophage-positive isolates, respectively. However, between 2002 and 2009 only 21 (12%) of the MDR *C. coli* isolates were from company B and E.

We analyzed 26 MDR *C. coli* isolates from 2002 - 2004 using *hip* primers and *CJE1461* primers. Only seven isolates were prophage-positive when tested with *hip* primers, but the numbers more than doubled (n=15), when they were analyzed with *CJI1461* primers (Table 3.1).

We also tested with *CJE1461* primers 36 *C. coli* isolates that were sensitive to at least two of the seven antimicrobial agents that we used in our laboratory; ampicilin, kanamycin, streptomycin, tetracycline, erythromycin, nalidixic acid and ciprofloxacin. The total number of prophage-positive isolates was only 10 (27.8 %). The results using *CJE1461* primers were considered consistent as shown from repeated tests on isolates SC2564, SC2666 and SC2761.

The MDR isolates that were positive with *hip* primers were also positive when analyzed by *CJE1461* primers (Figure 3.3). However certain isolates that were previously found negative with *hip* primers produced strong positive reaction with *CJE1461* primers (Figure 3.4). We never observed any positive prophage results from our control strains, *C. jejuni* ATCC 33560 and *C. jejuni* NTCT 11168.

Comparison with genotyping data. Analysis of dendrograms generated by BioNumerics software for our 152 MDR *C. coli* isolates revealed that the genotypes of prophage-positive and prophage-negative isolates were generally different from each other (Figure 3.8). From 75 different profiles generated based on PFGE with *Sma*I and *fla* typing, only two profiles (profiles 32 and 42) were found in both prophage-positive and negative isolates. The remaining 73 profiles were either for prophage-negative isolates or for prophage-positive isolates. The majority of the prophage-negative MDR *C. coli* isolates (n=27/46) were located in one big cluster that contain profiles 31 to 45, and the ST appeared

to be ST1126. We also observed that 10 prophage-negative isolates were located in two small clusters. These clusters contain profiles 50 and 51, and profiles 74 and 75, respectively.

3.5. DISCUSSION

We have successfully sequenced DNA fragments from two MDR *C. coli* isolates (SC2004 and SC1518) that were previously amplified by *hip* and *CJE1461* primers. The DNA sequences were identical with DNA sequenced from three *C. coli* isolates that were previously obtained in our laboratory using the *hip* primers. A check with the NCBI database showed that the sequence of the DNA fragment that we identified in our MDR *C. coli* were identical over at least 320 nucleotides to the *CJE1461* gene of *C. jejuni* RM1221 strain (GenBank accession number NC 0023912).

The *CJE1461* gene consists of a single open reading frame of 5700 bp that encodes a polypeptide of 1899 amino acids (GenBank accession number YP 179447). The function of the protein expressed from this gene is still unknown and is only referred to as hypothetical protein CJE1461. The location of the *CJE1461* gene in *C. jejuni* RM1221 is between nucleotide 11357767 and 1363466. Based on the study by Parker *et al*. (2006), the *CJE1461* gene was located in the region of the integrated element CJIE4 that has several genes predicted to encode phage-related proteins such as endonucleases, methylases, or repressor. The integrated element CJIE4 in *C. jejuni* RM1221 was found to have some similarity with the putative prophage contained in *C. lari* element 1 (CLIE1) within the *C. lari* RM2100 genome (Fouts *et al*., 2005).

Our DNA sequences were matched with the *CJE1461* gene sequence found in the NCBI database at nucleotides 2268 to 2400 for the *CJE1461* primer product, and at nucleotides 3956 to 4271 for the *hip* primer product. This suggested that the CJE1461 as described in NCBI database was present in a majority of our *C. coli* isolates.

Little is known about the function of CJE1461 in *Campylobacter*. Recently it was identified as "putative lysogenic bacteriophage that targeted tRNA-Met-1". More studies need to be carried out to determine the function of this gene in *Campylobacter*.

The results from our MLST analysis showed that there is no change in the clonal population of *C. coli* in comparison to what we have observed in 2002 to 2004 (D'lima *et al.*, 2007). However, we found that almost all (98%) of the prophage-positive isolates have genotypes different from isolates that were prophage-negative. The difference were more significantly observed in PFGE patterns with *Sma*I, suggesting that the presence of CJE1461 had contributed to the development of new PFGE profiles among MDR *C. coli* isolates.

We have shown the increase in prevalence of *CJE1461* gene in our MDR *C. coli* from 26.9% for isolates received between 2002 and 2004 to 81% for isolates from 2009. The numbers that we identified were based on the results of PCR using *hip* primers. There is a possibility that more MDR *C. coli* harbor this gene as has been demonstrated in isolates SC3018 and SC3021, which were negative with *hip* primers but positive with the *CJE1461* primers.

We also showed that the prevalence of prophage-positive isolates in MDR *C. coli* was much higher (63%; Table 3.3) compared to prevalence in non-MDR *C. coli* (33.3% Table 3.2). Even when we compared the results from non-MDR *C. coli* isolates using the more

specific *CJE1461* primers, the prevalence of prophage-positive isolates was only 27% (n=10/36) (Table 3.3). These data suggest that the product of *CJE1461* gene could have contributed to the prevalence of MDR *C. coli* strains. Further study is needed to determine the sudden increase in prevalence of the prophage gene in MDR *C. coli* in turkey flocks and how this *CJE1461* gene may have affected the genetic structure, virulence and ability of *C. coli* to colonize turkeys.

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Table 3.1. Results from PCR amplification using *hip* primers and *CJE1461* primers on multidrug resistance *C. coli* isolated from 2002 to 2009.

No.	Isolates	Received	Company	ST[1]	*Hip*	CJE1461
1	SC182	4/9/2003	A	1101	-	+
2	SC280	7/8/2003	D	1101	-	+
3	1788	5/7/2002	B	1101	-	-
4	5988	11/14/2003	D	1101	+	+
5	6685	5/17/2004	B	1101	-	+
6	6818	6/7/2004	D	1101	+	+
7	6851	6/10/2004	B	1101	+	+
8	7135	6/30/2004	B	1101	-	+
9	7144	6/30/2004	B	1101	-	+
10	7221	7/6/2004	D	1101	+	+
11	7432	7/15/2004	B	1101	-	+
12	8325	9/15/2004	D	1149	+	+
13	8635	10/6/2004	D	2934	-	+
14	7877	8/9/2004	D	1126	-	-
15	SC355	7/9/2003	A	1126	-	-
16	8002	8/11/2004	B	1126	+	+
17	6840	6/9/2004	D	1126	-	-
18	SC144	4/9/2003	C	1154	-	-
19	5991	11/14/2003	D	1154	-	-
20	SC242	7/8/2003	D	1170	-	-
21	8260	9/8/2004	D	1198	-	-
22	6958	6/21/2004	D	1171	-	-
23	6282	3/25/2004	D	889	-	+
24	6449	4/8/2004	D	1149	-	-
25	6605	5/4/2004	D	1149	+	+
26	7156	6/30/2004	D	889	-	-
27	SC1870	6/28/2007	B		-	-
28	SC1444	12/13/2006	C		-	-
29	SC2004	1/16/2008	C		+	+
30	SC2009	1/16/2008	C		+	+
31	SC1994	1/15/2008	D		-	-
32	SC2565	6/16/2009	D		+	+
33	SC2595	6/16/2009	D		+	+

[1] The STs were obtained from D'lima *et al*. (2007).

Table 3.1. Continued.

No.	Isolates	Received	Company	ST[1]	*Hip*	CJE1461
34	SC2780	12/1/2009	D		+	+
35	SC2800	12/1/2009	D		+	+
36	SC2818	12/1/2009	D		+	+
37	SC2835	12/1/2009	D		+	+
38	SC2843	12/2/2009	C		+	+
39	SC2849	12/2/2009	C		-	+
40	SC2893	12/2/2009	C		-	+
41	SC2895	12/2/2009	A		-	+
42	SC2909	12/2/2009	A		+	+
43	SC2916	12/2/2009	A		+	+
44	SC2923	12/2/2009	A		+	+
45	SC2924	12/2/2009	A		+	+
46	SC2937	12/2/2009	A		+	+
47	SC2944	12/2/2009	A		+	+
48	SC2984	12/3/2009	B		+	+
49	SC3018	12/9/2009	E		-	+
50	SC3021	12/9/2009	E		-	+

Table 3.2. Test on 36 *C. coli* isolates that are sensitive to at least one type of antimicrobial agent (tetracycline, streptomycin, erythromycin, kanamycin, nalidixic acid, or ciprofloxacin) using *hip* primers and *CJE1461* primers. Only 10 isolates were positive when analyzed by *CJE1461* primers. Only 9 isolates were analyzed with *hip* primers and 3 were positive.

ID	Date Received	Company	Antimicrobial agents						*Hip*	CJE1461
			Tet-10*	Str-15*	E-10*	K-25*	NA-20*	Cip-4*		
SC2564	6/16/2009	D	+	-	-	-	-	-	+	+ [a]
SC2582	6/16/2009	D	+	-	-	-	+	+	-	-
SC2602	6/16/2009	D	+	-	-	-	+	+	-	-
SC2699	6/24/2009	E	+	-	-	+	-	-	-	-
SC2720	6/25/2009	B	+	-	-	+	+	+	+	+
SC2723	6/25/2009	B	+	-	-	+	+	+	+	+
SC2750	6/25/2009	B	+	-	-	+	+	+	-	-
SC2585	6/16/2009	D	+	-	-	-	-	-	NA	-
SC2590	6/16/2009	D	+	-	-	-	+	+	NA	-
SC2601	6/16/2009	D	+	-	-	-	+	+	NA	-
SC2666	6/17/2009	A	-	-	-	+	-	-	-	- [a]
SC2701	6/24/2009	E	+	-	-	+	-	-	NA	-
SC2761	6/25/2009	B	+	-	-	-	+	+	-	- [a]
SC2783	12/1/2009	D	-	+	+	+	-	-	NA	+
SC2820	12/1/2009	D	+	-	-	-	+	+	NA	-
SC2852	12/2/2009	C	+	-	-	-	+	+	NA	-
SC2866	12/2/2009	C	+	-	-	-	+	+	NA	-
SC2883	12/2/2009	C	+	-	-	+	-	-	NA	+
SC2886	12/2/2009	C	-	-	-	-	+	+	NA	+
SC2900	12/2/2009	A	+	-	-	-	+	+	NA	-
SC2905	12/2/2009	A	+	-	-	-	+	+	NA	-
SC2913	12/2/2009	A	-	+	+	+	+	+	NA	-
SC2929	12/2/2009	A	+	-	-	+	-	-	NA	+
SC2941	12/2/2009	A	+	-	-	+	-	-	NA	+
SC2942	12/2/2009	A	+	-	-	-	+	+	NA	-
SC2979	12/3/2009	B	+	-	-	-	+	+	NA	-
SC2999	12/9/2009	E	+	-	-	+	-	-	NA	-
SC3001	12/9/2009	E	+	-	-	+	-	-	NA	-
SC3037	2/16/2010	D	+	-	-	-	+	+	NA	-
SC3134	2/17/2010	C	+	-	-	-	+	+	NA	+
SC3163	2/17/2010	A	+	-	-	+	-	-	NA	+
SC3269-1	5/11/2010	D	+	-	-	-	+	+	NA	-
SC3287-2	5/11/2010	D	+	-	-	-	+	+	NA	-
SC3366	5/12/2010	C	-	-	-	-	-	-	NA	-
SC3368	5/11/2010	C	-	+	-	-	-	-	NA	-
SC3369	5/12/2010	C	-	-	-	-	-	-	NA	-

Abbreviation: Tet – tetracycline, Str – streptomycin, E – erythromycin, K – kanamycin, NA – nalidixic acid, Cip – ciprofloxacin.

***** The numbers represent the concentration of antimicrobial agent in μg/ml.

[a] These isolates were analyzed two times with *CJE1461* primers and produced similar results.

Table 3.3. The results of prophage test for MDR *C. coli* isolates from five different companies received between 2006 and 2009. The numbers in the parenthesis indicate the percentage of the positive and negative results from the total number in each company at the respective year.

Year	Result from *hip* primers for Companies A - E										Total	
	A		B		C		D		E			
	+	-	+	-	+	-	+	-	+	-	+	-
2002-2004	0	2	2	5	0	1	5	11	0	0	7 (27%)	19 (73%)
2006	4 (44%)	5 (565%)	0	0	4 (50%)	4 (50%)	0	0	0	0	8 (47%)	9 (53%)
2007	13 (72%)	5 (28%)	0 (0%)	1 (100 %)	3 (50%)	3 (50%)	20 (67%)	10 (33%)	0	0	36 (65%)	19 (35%)
2008	9 (75%)	3 (25%)	2 (100%)	0 (0%)	8 (89%)	1 (11%)	9 (60%)	6 (40%)	0	0	28 (74%)	10 (26%)
2009	16 (100 %)	0 (0%)	2 (29%)	5 (71%)	6 (100%)	0 (0%)	8 (89%)	1 (11%)	2 (50%)	2 (50%)	34 (81%)	8 (19%)
Total	42 (74%)	15 (26%)	6 (35%)	11 (65%)	21 (70%)	9 (30%)	42 (60%)	28 (40%)	2 (50%)	2 (50%)	113 (63%)	65 (37%)

```
CLUSTAL 2.0.12 multiple sequence alignment


CJE1461-C. jejuni  ACCTACTATAGTTCCAATGCCAGGCATTATAATACTACCTAATGCACCACCTATCGCACC 2220
Primer_CJE1461_F   ------------------------------------------------------CGCACC 6
                                                                        ******

CJE1461-C. jejuni  ACCTGTTCCTGCGTGAGTATCAGCCTTAAAAAGCCAATCACCTAAGCTTCCTATGCCATA 2280
Primer_CJE1461_F   ACCTGTTCCTGCGT---------------------------------------------- 20
                   **************

CJE1461-C. jejuni  ACCTAGAAGTCCGCCCATTGCTGCATTAGCTAAAGTGCCACCTATATAGCCCAAGCCCCC 2340
Primer_CJE1461_R   ------------------------------------------------------------


CJE1461-C. jejuni  ACCAAAACCAAGAGATAAAGCATTACTTGCACCACTTAATCCCATACCCGCCAAAGAAGC 2400
Primer_CJE1461_R   ----------------------------------------------ACCCGCCAAAGAAGC 15
                                                                 ***************

CJE1461-C. jejuni  ACCGTTTGTAAAGCTTGCACCAAGTCCTAAAAAATTTGCCAAATAAGCTGAACCATTAGC 2460
Primer_CJE1461_R   ACCGT------------------------------------------------------ 20
                   *****
```

Figure 3.1. Sequence comparison of nucleotide between *CJE1461* F primer (CGCACCACCTGTTCCTGCGT) and the complimentary DNA sequence of *CJE1461* R primer (ACGGTGCTTCTTTGGCGGGT) that was developed from our lab (unpublished data) with the *CJE1461* gene from *C. jejuni* RM1221 (5700 bp). The nucleotide of *CJE1461* primer set was matched with the *CJE1461* gene sequence at nucleotide from 2215 to 2234, and 2386 to 2405, respectively. This primer should amplified 230 bp DNA from this gene.

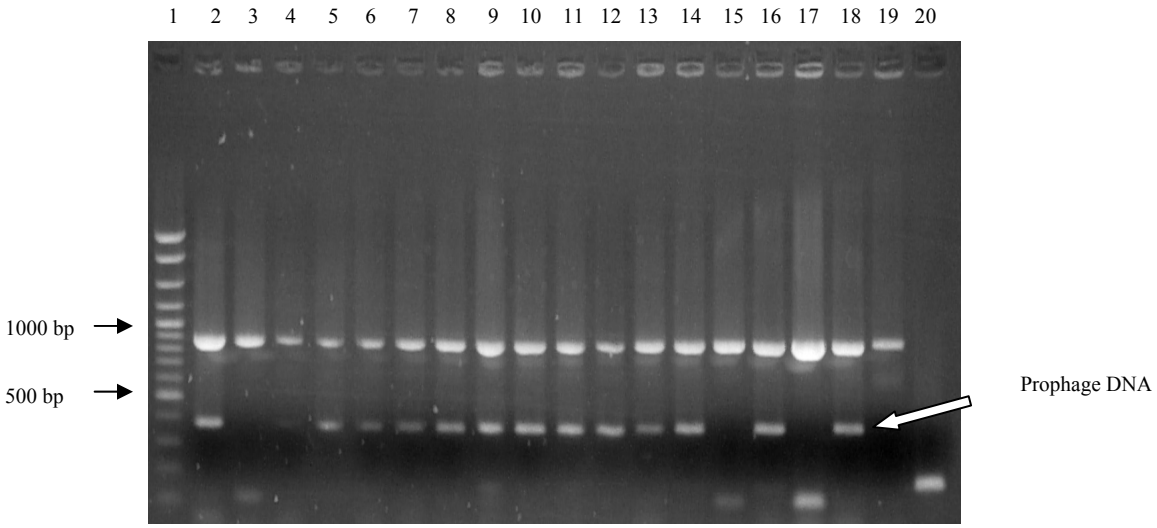


Figure 3.2. MDR *C. coli* isolates analyzed with mixed primers (*hip* primer and *ceu*E primer). Lane 1, Molecular weight; lane 1, 5 – 14 were MDR *C. coli* isolates that show the presence of prophage DNA; lane 3, 4, 15, and 17 were MDR *C. coli* that were considered prophage negative; lane 19 is *C. coli* D124, lane 20 is *C. jejuni* ATCC 33560. The size of prophage DNA amplified in this reaction is approximately 380 bp.

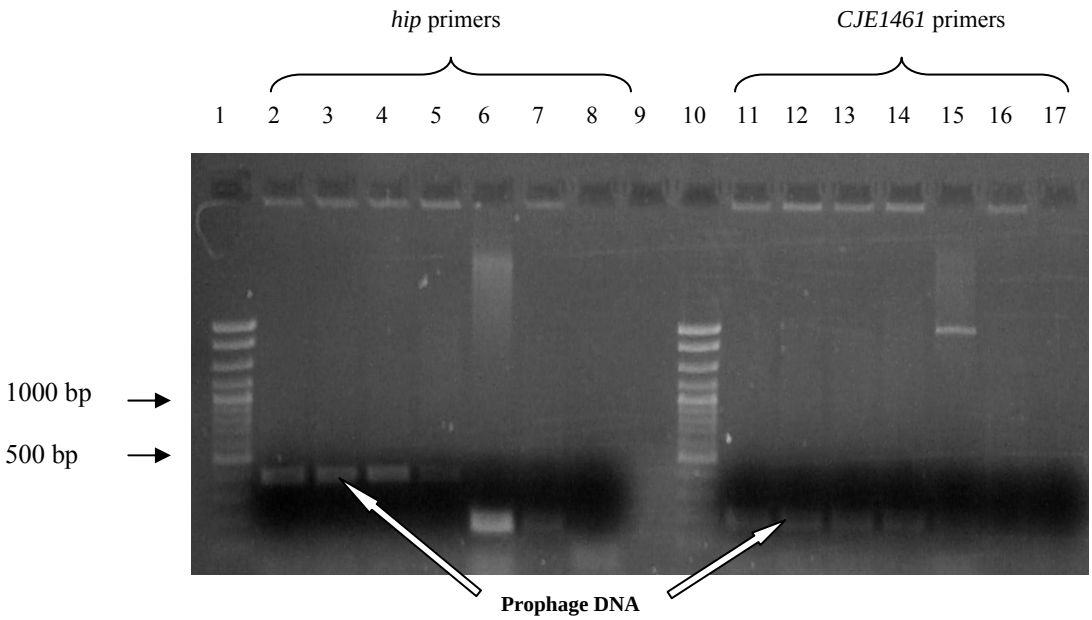


Figure 3.3. Gel electrophoresis showed the results of PCR reaction using *hip* primers and *CJE1461* primer on the same MDR *C. coli* isolates. The size of prophage DNA amplified by *hip* primers is approximately 380 bp while the *CJE1461* primers only generate about 230 bp DNA fragment. Lane 1 and 10 were the standard molecular weight DNA, lane 2 – 5 and 11 – 14 contain the MDR C. coli of the same isolates, lane 6 and 15 were *C. jejuni* NTCT 11168, lane 7 and 16 were *C. jejuni* ATCC 33560, lane 8 and 17 were blank test, and lane 9 was empty.

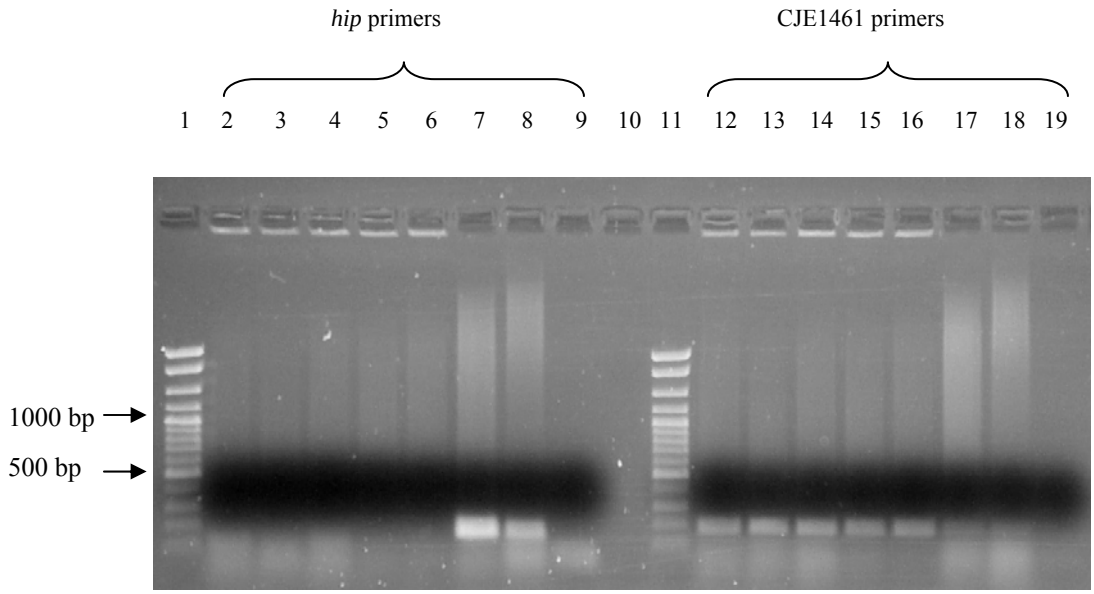


Figure 3.4. Gel electrophoresis showed the results of PCR reaction using *hip* primers and *CJE1461* primer on the same MDR *C. coli* isolates. The was no DNA amplified by *hip* primers from all the MDR *C. coli* isolates (lane 2 – 6) while the *CJE1461* primers had produced positive prophage band (lane 12 – 16) with size about 230 bp nucleotides. Lane 1 and 11 were the standard molecular weight, Lane 7 and 17 were *C. jejuni* NTCT 11168, Lane 8 and 18 were *C. jejuni* ATCC 33560, Lane 9 and 19 were blank test, and lane 10 was empty.

```
CLUSTAL 2.0.12 multiple sequence alignment

SC1783-Hip1a      TCGTAGCTTTTCTAGCTTCTTTATTCATTTTTAGCTGTTTTCCGATAACACTTTCAAACA 60
SC1821-Hip1a      TCGNAGCTTTTCTAGCTTCTTTATTCATTTTTAGCTGTTTTCCGGTAACACTTTCAAACA 60
SC1800-Hip1a      -CGNANCTTTTCTAGCTTCTTTATTCNTTTTTAGCTGTTTTCCGGTAACACTTTCAAACA 59
SC2004-hip1a      ------CTTTTCTAGCTTCTTTATTCATTTTTAGCTGTTTTCCGGTAACACTTTCAAACA 54
SC1585-hip1a      ------CTTNNCTAGCTTCTTTATTCATTTTTAGCTGTTTTCCGGTAACACTTTCAAACA 54
                        ***  *************** ***************** ***************

SC1783-Hip1a      TATAATCAGTGCCTTTTAAAACACTTTCTACACCTTCTTTTATATGCCAAAAATCTAATT 120
SC1821-Hip1a      TATAATCAGTGCCTTTTAAAACACTTTCTACACCTTCTTTTATATGCCAAAAATCTAATT 120
SC1800-Hip1a      TATAATCAGTGCCTTTTAAAACACTTTCTACACCTTCTTTTATATGCCAAAAATCTAATT 119
SC2004-hip1a      TATAATCAGTGCCTTTTAAAACACTTTCTACACCTTCTTTTATATGCCAAAAATCTAATT 114
SC1585-hip1a      TATAATCAGTGCCTTTTAAAACACTTTCTACACCTTCTTTTATATGCCAAAAATCTAATT 114
                  ************************************************************

SC1783-Hip1a      TTGATGTGATTTGAGCTCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTT 180
SC1821-Hip1a      TTGATGTGATTTGAGCTCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTT 180
SC1800-Hip1a      TTGATGTGATTTGAGCTCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTT 179
SC2004-hip1a      TTGATGTGATTTGAGCTCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTT 174
SC1585-hip1a      TTGATGTGATTTGAGCTCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTT 174
                  ************************************************************

SC1783-Hip1a      TAAGATTTTTAACATACTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTG 240
SC1821-Hip1a      TAAGATTTTTAACATACTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTG 240
SC1800-Hip1a      TAAGATTTTTAACATACTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTG 239
SC2004-hip1a      TAAGATTTTTAACATACTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTG 234
SC1585-hip1a      TAAGATTTTTAACATACTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTG 234
                  ************************************************************

SC1783-Hip1a      ATGTTTTTTCTAAAGCTTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAG 300
SC1821-Hip1a      ATGTTTTTTCTAAAGCTTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAG 300
SC1800-Hip1a      ATGTTTTTTCTAAAGCTTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAG 299
SC2004-hip1a      ATGTTTTTTCTAAAGCTTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAG 294
SC1585-hip1a      ATGTTTTTTCTAAAGCTTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAG 294
                  ************************************************************

SC1783-Hip1a      TCATCAAAGCTATAAGCCCAGCAGTTGTAA------------ 330
SC1821-Hip1a      TCATCAAAGCTATAAGCCCAGCAGTTGTAA------------ 330
SC1800-Hip1a      TCATCAAAGCTATAAGCCCAGCAGTTGTAANNANAGNANN-- 339
SC2004-hip1a      TCATCAAAGCTATAAGCCCAGCAGTTGTAAGCA-TAGGAG-- 333
SC1585-hip1a      TCATCAAAGCTATAAGCCCAGCAGTTGTAAGNAATAGGAGCN 336
                  ****************************
```

Figure 3.5. Sequence comparison between our *C. coli* isolates (SC2004 and SC1585) and *C. coli* from our previous unpublished data amplified by *hip* primers. SC1783 (354 bp), SC1821 (352 bp), and SC1800 (347 bp) were also isolated from turkey in 2007. SC1783 and SC1821 were MDR strains from company D and A, respectively. SC1800 was isolated from company C and was found to be resistant to tetracycline, kanamycin, nalidixic acid, and ciprofloxacin, but sensitive to streptomycin and erythromycin. The DNA sequences were between 330 and 336 nucleotides were considered matched to each other, except in nucleotide number 44.

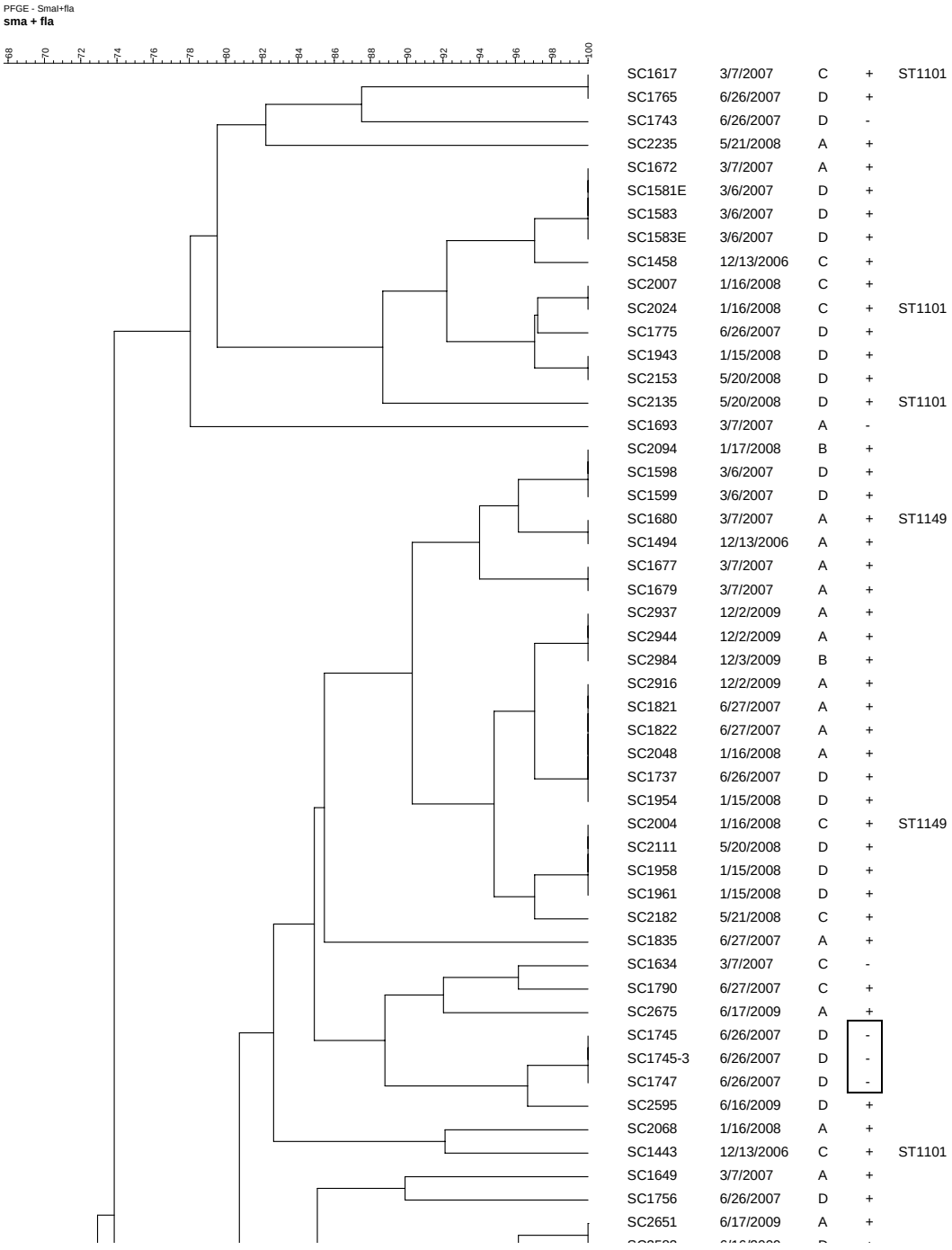
```
CLUSTAL 2.0.12 multiple sequence alignment


CJE1461 gene          TTTGGCGGCTTCTTCGGCTAGTTTTAAATTTGCCTGTATTTGTTCGTAAGCTTTTCTAGC 3960
SC2004-hip1A          ----------------------------------------------------TTTTCTAGC 9
SC1585-hip1A          -------------------------------------------GTAN-CTTNNCTAGC 14
                                                                    **  *****

CJE1461 gene          TTCTTTATTCATTTTTAGCTGTTTTCCGGTAACACTTTCAAACATATAATCAGTGCCTTT 4020
SC2004-hip1A          TTCTTTATTCATTTTTAGCTGTTTTCCGGTAACACTTTCAAACATATAATCAGTGCCTTT 69
SC1585-hip1A          TTCTTTATTCATTTTTAGCTGTTTTCCGGTAACACTTTCAAACATATAATCAGTGCCTTT 74
                      ************************************************************

CJE1461 gene          TAAAACACTTTCTACACCTTCTTTTATATACCAAAAATCTAATTTTGATGTGATTTGAGC 4080
SC2004-hip1A          TAAAACACTTTCTACACCTTCTTTTATATGCCAAAAATCTAATTTTGATGTGATTTGAGC 129
SC1585-hip1A          TAAAACACTTTCTACACCTTCTTTTATATGCCAAAAATCTAATTTTGATGTGATTTGAGC 134
                      *************************** ****************************

CJE1461 gene          TCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTTTAAGATTTTTAACATA 4140
SC2004-hip1A          TCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTTTAAGATTTTTAACATA 189
SC1585-hip1A          TCCCAAAACACTTCCATTTGCTTCTAAGGCATCCATTTGACTTTTAAGATTTTTAACATA 194
                      ************************************************************

CJE1461 gene          CTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTGATGTTTTTTCTAAAGC 4200
SC2004-hip1A          CTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTGATGTTTTTTCTAAAGC 249
SC1585-hip1A          CTCATCTAATTCTTTTGAACTTTTGTTTTCTAGCTTTCTTTTTGATGTTTTTTCTAAAGC 254
                      ************************************************************

CJE1461 gene          TTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAGTCATCAAAGCTATAAG 4260
SC2004-hip1A          TTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAGTCATCAAAGCTATAAG 309
SC1585-hip1A          TTTGTCAAAATTATCCCAATTCGTATAAAGCTTTTCTATAAAAGTCATCAAAGCTATAAG 314
                      ************************************************************

CJE1461 gene          CCCAGCAGTTGGAGCTAAAGCCTTTGTAAAATTTAAAGCAGAATTTGCTAAATTACTCAT 4320
SC2004-hip1A          CCCAGCAGTTG----TAAG-----------------------------------CAT 327
SC1585-hip1A          CCCAGCAGTTG---------------------------------------------- 325
                      ***********

CJE1461 gene          AGTGCCTTTTATGGTGCTTAACAAAGGATTTCTTTTTAAGATACTTTCATTAATTAAAGT 4380
SC2004-hip1A          AG--------------------GAG---------------------------------- 332
SC1585-hip1A          ----------------------------------TAAGN------------------- 330
```

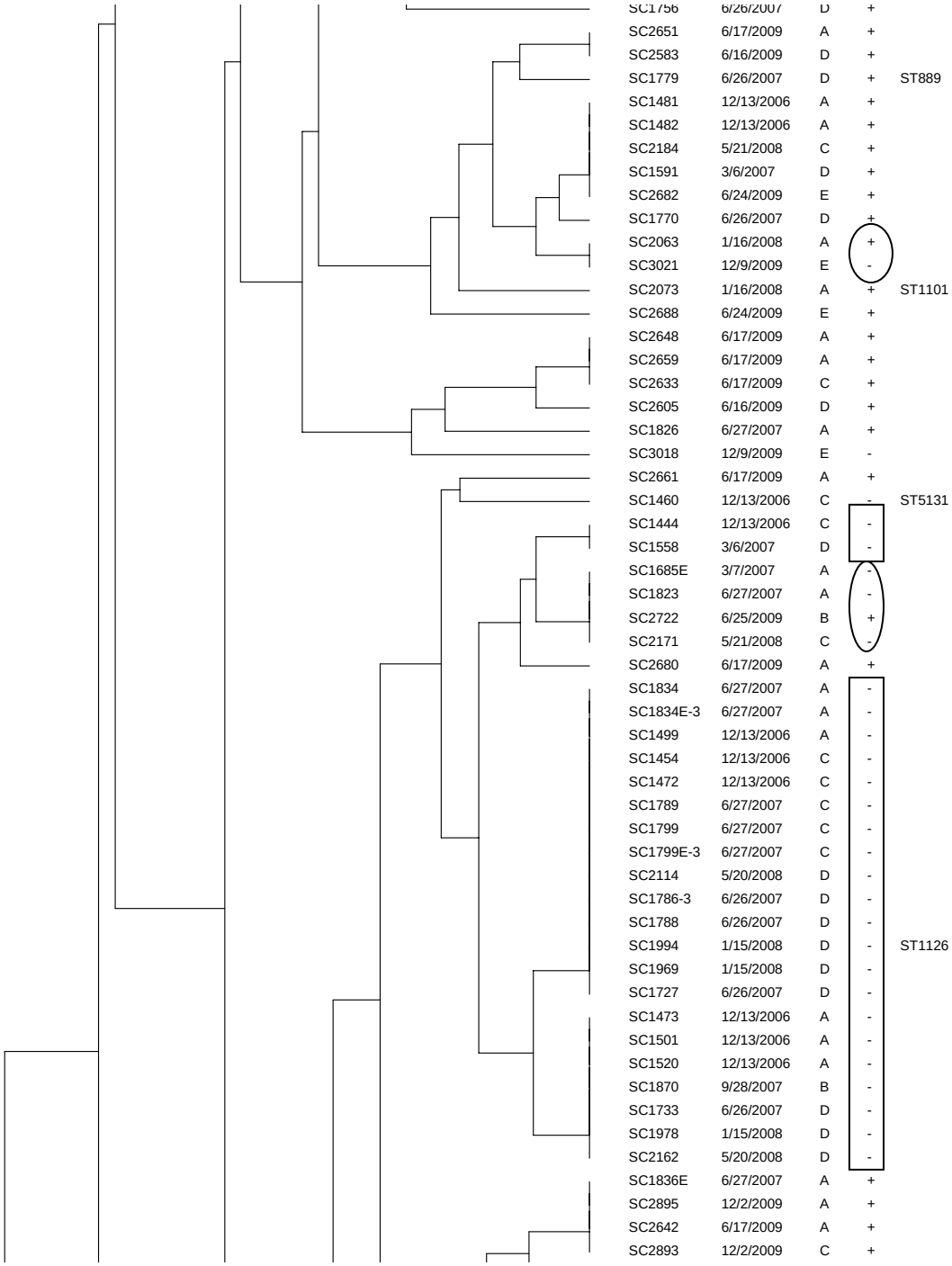
Figure 3.6. The comparison between DNA amplified by *hip* primers and *CJE1461* gene in *C. jejuni* RM1221. The sizes of both amplicons were about 320 bp and were matched at nucleotide between 3956 and 4271 in *CJE1461* gene. The 5.70 kb DNA sequence of *CJE1461* gene that can be found in *C. jejuni* RM1221 genome from base 1357767 to 1363466. The complete sequence can be found in NCBI database with Accession number: NC 0023912 (http://www.ncbi.nlm.nih.gov/nuccore/57236892?from=1357767&to=1363466&report=gbwithparts)

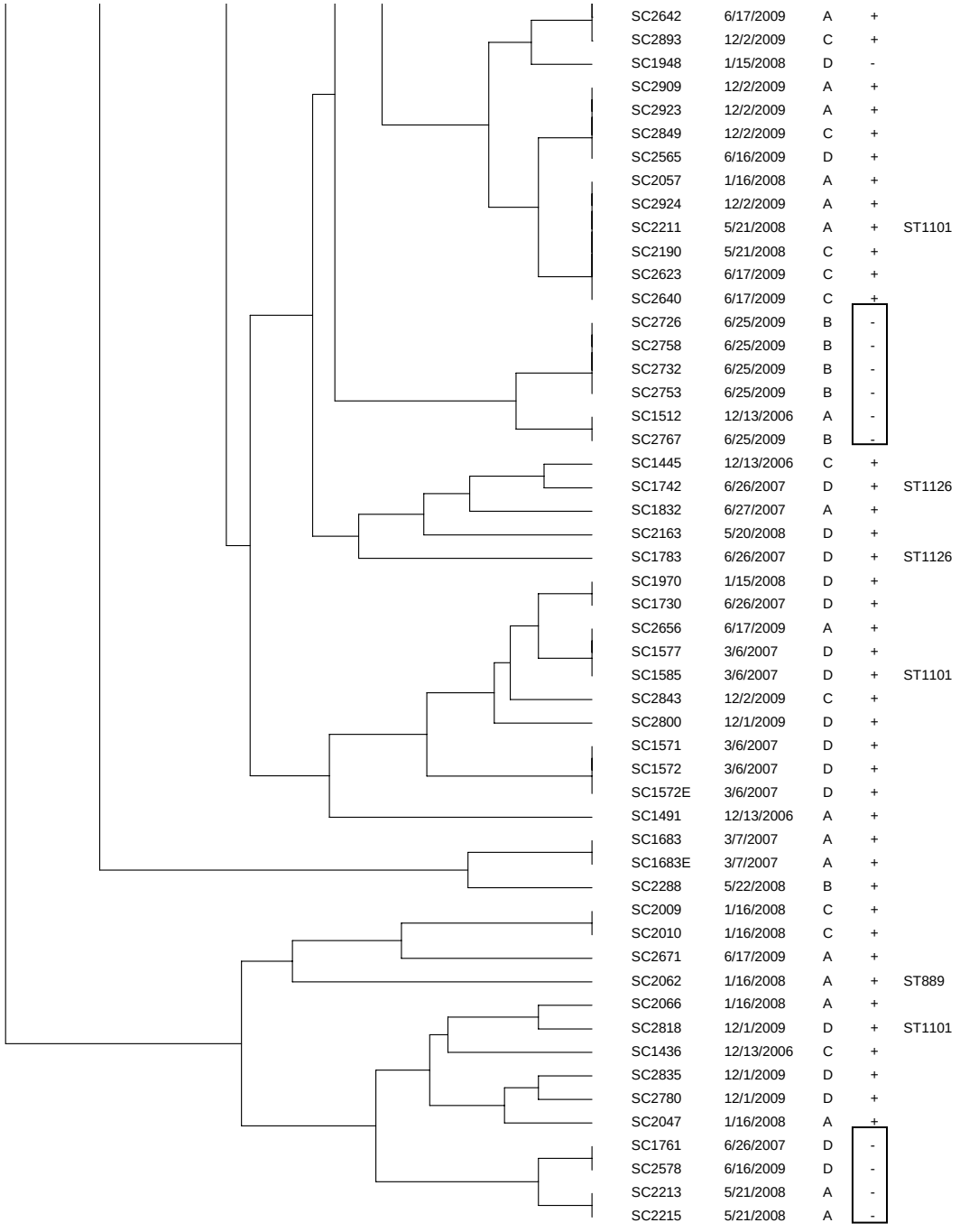
```
CLUSTAL 2.0.12 multiple sequence alignment


CJE1461 gene        AATGCACCACCTATCGCACCACCTGTTCCTGCGTGAGTATCAGCCTTAAA 2250
SC1585-CJE1461F     --------------------------------------------------
SC2004-CJE1461F     --------------------------------------------------


CJE1461 gene        AAGCCAATCACCTAAGCTTCCTATGCCATAACCTAGAAGTCCGCCCATTG 2300
SC1585-CJE1461F     ---------------GCTTCCTATGCCATAACCTAGAAGTCCGCCCATTG 35
SC2004-CJE1461F     -----------------TTCCTATGCCNTAACCTAGAAGTCCGCCCATTG 33
                                     ********** ********************** 

CJE1461 gene        CTGCATTAGCTAAAGTGCCACCTATATAGCCCAAGCCCCCACCAAAACCA 2350
SC1585-CJE1461F     CTGCATTAGCTAAAGTGCCACCTATATAGCCCAAGCCCTCACCAAAACCA 85
SC2004-CJE1461F     CTGCATTAGCTAAAGTGCCACCTATATAGCCCAAGCCCTCACCAAAACCA 83
                    ************************************ ***********

CJE1461 gene        AGAGATAAAGCATTACTTGCACCACTTAATCCCATACCCGCCAAAGAAGC 2400
SC1585-CJE1461F     AGAGATAAAGCATTACTTGCACCACTTAATCCCATACCCGCCAAAGAAGC 135
SC2004-CJE1461F     AGAGATAAAGCATTACTTGCACCACTTAATCCCATACCCGCCAAAGAAGC 133
                    ************************************************

CJE1461 gene        ACCGTTTGTAAAGCTTGCACCAAGTCCTAAAAAATTTGCCAAATAAGCTG 2450
SC1585-CJE1461F     --------------------------------------------------
SC2004-CJE1461F     ACCGT---------------------------------------AACNG 143
```

Figure 3.7. The comparison of DNA amplified by *CJE1461* F primers on two MDR *C. coli* isolates (SC2004 and 6SC1585)and *CJE1461* gene from *C. jejuni* RM1221.

Figure 3.8. Localization of prophage positive and prophage negative MDR *C. coli* isolates in *flaA* and PFGE based dendrogram. The majority of the negative prophage isolates were clustered among each other (rectangular box), and only in 2 clusters where the positive and negative prophage isolates have identical *flaA* and PFGE pattern (circle shape). Sequence types (ST) were previously determined in Chapter II for eight isolates.


PFGE - SmaI+fla
sma + fla
68 70 72 74 76 78 80 82 84 86 88 90 92 94 96 98 100
SC1617 3/7/2007 C + ST1101
SC1765 6/26/2007 D +
SC1743 6/26/2007 D -
SC2235 5/21/2008 A +
SC1672 3/7/2007 A +
SC1581E 3/6/2007 D +
SC1583 3/6/2007 D +
SC1583E 3/6/2007 D +
SC1458 12/13/2006 C +
SC2007 1/16/2008 C +
SC2024 1/16/2008 C + ST1101
SC1775 6/26/2007 D +
SC1943 1/15/2008 D +
SC2153 5/20/2008 D +
SC2135 5/20/2008 D + ST1101
SC1693 3/7/2007 A -
SC2094 1/17/2008 B +
SC1598 3/6/2007 D +
SC1599 3/6/2007 D +
SC1680 3/7/2007 A + ST1149
SC1494 12/13/2006 A +
SC1677 3/7/2007 A +
SC1679 3/7/2007 A +
SC2937 12/2/2009 A +
SC2944 12/2/2009 A +
SC2984 12/3/2009 B +
SC2916 12/2/2009 A +
SC1821 6/27/2007 A +
SC1822 6/27/2007 A +
SC2048 1/16/2008 A +
SC1737 6/26/2007 D +
SC1954 1/15/2008 D +
SC2004 1/16/2008 C + ST1149
SC2111 5/20/2008 D +
SC1958 1/15/2008 D +
SC1961 1/15/2008 D +
SC2182 5/21/2008 C +
SC1835 6/27/2007 A +
SC1634 3/7/2007 C -
SC1790 6/27/2007 C +
SC2675 6/17/2009 A +
SC1745 6/26/2007 D -
SC1745-3 6/26/2007 D -
SC1747 6/26/2007 D -
SC2595 6/16/2009 D +
SC2068 1/16/2008 A +
SC1443 12/13/2006 C + ST1101
SC1649 3/7/2007 A +
SC1756 6/26/2007 D +
SC2651 6/17/2009 A +


SC1756 6/26/2007 D +
SC2651 6/17/2009 A +
SC2583 6/16/2009 D +
SC1779 6/26/2007 D + ST889
SC1481 12/13/2006 A +
SC1482 12/13/2006 A +
SC2184 5/21/2008 C +
SC1591 3/6/2007 D +
SC2682 6/24/2009 E +
SC1770 6/26/2007 D +
SC2063 1/16/2008 A +
SC3021 12/9/2009 E -
SC2073 1/16/2008 A + ST1101
SC2688 6/24/2009 E +
SC2648 6/17/2009 A +
SC2659 6/17/2009 A +
SC2633 6/17/2009 C +
SC2605 6/16/2009 D +
SC1826 6/27/2007 A +
SC3018 12/9/2009 E -
SC2661 6/17/2009 A +
SC1460 12/13/2006 C - ST5131
SC1444 12/13/2006 C -
SC1558 3/6/2007 D -
SC1685E 3/7/2007 A -
SC1823 6/27/2007 A -
SC2722 6/25/2009 B +
SC2171 5/21/2008 C -
SC2680 6/17/2009 A +
SC1834 6/27/2007 A -
SC1834E-3 6/27/2007 A -
SC1499 12/13/2006 A -
SC1454 12/13/2006 C -
SC1472 12/13/2006 C -
SC1789 6/27/2007 C -
SC1799 6/27/2007 C -
SC1799E-3 6/27/2007 C -
SC2114 5/20/2008 D -
SC1786-3 6/26/2007 D -
SC1788 6/26/2007 D -
SC1994 1/15/2008 D - ST1126
SC1969 1/15/2008 D -
SC1727 6/26/2007 D -
SC1473 12/13/2006 A -
SC1501 12/13/2006 A -
SC1520 12/13/2006 A -
SC1870 9/28/2007 B -
SC1733 6/26/2007 D -
SC1978 1/15/2008 D -
SC2162 5/20/2008 D -
SC1836E 6/27/2007 A +
SC2895 12/2/2009 A +
SC2642 6/17/2009 A +
SC2893 12/2/2009 C +


SC2642 6/17/2009 A +
SC2893 12/2/2009 C +
SC1948 1/15/2008 D -
SC2909 12/2/2009 A +
SC2923 12/2/2009 A +
SC2849 12/2/2009 C +
SC2565 6/16/2009 D +
SC2057 1/16/2008 A +
SC2924 12/2/2009 A +
SC2211 5/21/2008 A + ST1101
SC2190 5/21/2008 C +
SC2623 6/17/2009 C +
SC2640 6/17/2009 C +
SC2726 6/25/2009 B -
SC2758 6/25/2009 B -
SC2732 6/25/2009 B -
SC2753 6/25/2009 B -
SC1512 12/13/2006 A -
SC2767 6/25/2009 B -
SC1445 12/13/2006 C +
SC1742 6/26/2007 D + ST1126
SC1832 6/27/2007 A +
SC2163 5/20/2008 D +
SC1783 6/26/2007 D + ST1126
SC1970 1/15/2008 D +
SC1730 6/26/2007 D +
SC2656 6/17/2009 A +
SC1577 3/6/2007 D +
SC1585 3/6/2007 D + ST1101
SC2843 12/2/2009 C +
SC2800 12/1/2009 D +
SC1571 3/6/2007 D +
SC1572 3/6/2007 D +
SC1572E 3/6/2007 D +
SC1491 12/13/2006 A +
SC1683 3/7/2007 A +
SC1683E 3/7/2007 A +
SC2288 5/22/2008 B +
SC2009 1/16/2008 C +
SC2010 1/16/2008 C +
SC2671 6/17/2009 A +
SC2062 1/16/2008 A + ST889
SC2066 1/16/2008 A +
SC2818 12/1/2009 D + ST1101
SC1436 12/13/2006 C +
SC2835 12/1/2009 D +
SC2780 12/1/2009 D +
SC2047 1/16/2008 A +
SC1761 6/26/2007 D -
SC2578 6/16/2009 D -
SC2213 5/21/2008 A -
SC2215 5/21/2008 A -