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PII: S0168-1605(20)30150-1

DOI: https://doi.org/10.1016/j.ijfoodmicro.2020.108656

Reference: FOOD 108656

To appear in: *International Journal of Food Microbiology*

Received date: 13 July 2019

Revised date: 25 April 2020

Accepted date: 5 May 2020

Please cite this article as: A.R. Alaboudi, I.M. Malkawi, T.M. Osaili, et al., Prevalence, antibiotic resistance and genotypes of Campylobacter jejuni and Campylobacter coli isolated from chickens in Irbid governorate, Jordan, *International Journal of Food Microbiology* (2020), https://doi.org/10.1016/j.ijfoodmicro.2020.108656

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Prevalence, antibiotic resistance and genotypes of *Campylobacter jejuni* and *Campylobacter coli* isolated from chickens in Irbid governorate, Jordan

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Keywords: *Campylobacter jejuni*; *Campylobacter coli*; Chickens; Antibiotic Resistance, Slaughterhouses.

Abstract

Campylobacter is the world's leading cause of bacterial gastroenteritis, causing nearly 9 million cases of food poisoning in Europe every year. Poultry is considered the main source of *Campylobacter* infection to humans. The objectives of the study were to determine occurrence of *C. jejuni* and *C. coli* in chickens, the antimicrobial resistance, genotypes, and relatedness of the isolates. A total of 177 chicken samples obtained from informal butcher shops (fresh), formal poultry slaughterhouses (refrigerated) and retail market (frozen) were analyzed. Isolation of *Campylobacter* spp. was conducted according to the ISO 10272-2006 method. Multiplex PCR was used for confirmation and identification of the isolates. The disk diffusion method was used to determine the antimicrobial resistance of the isolates and multilocus sequence typing was used for genotyping. The proportion of samples with *Campylobacter* spp. was 31.6% among all chicken samples (fresh and refrigerated 47.5%, frozen 0%) *C. coli* was isolated from 42.4% of chicken samples obtained from butcher shops and from 18.6% of samples obtained in formal slaughterhouses. *C. jejuni* was isolated from 17.0% of samples obtained in butcher shops and formal slaughterhouses. *Campylobacter* spp. was not isolated in frozen chicken samples. All tested isolates showed resistance towards ciprofloxacin and susceptibility toward imipenem and all of the isolates were multidrug resistant toward 5 or more antimicrobials. Three sequence types were identified among 10 *C. coli* isolates and seven sequence types were identified among 10 *C. jejuni* isolates. Among sequence types, chicken isolates shared similarities of both phenotypic and genetic levels.

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1. Introduction

Campylobacteriosis is a disease caused by members of *Campylobacter* sp. In low and middle income countries (LMICs) infection in toddlers is mainly caused by *C. jejuni* (Oberhelman, 2000), although *C. coli*, *C. fetus* and *C. upsaliensis* can also infect humans. Ingestion of only 500 cells may cause acute diarrhea to humans (Whiley et al., 2013).

All poultry species can carry *Campylobacter*; however, chickens pose a greater risk to humans due to the frequency of consumption (Humphrey et al., 2007). Consumption of raw or undercooked chicken or cross-contaminated from raw chicken can result in clinical cases (Nadeau et al., 2002). There is always a potential risk of contamination of chicken meat with *Campylobacter*, if hygienic measures are not applied properly during processing (Osaili et al., 2012). In Jordan, butcher shops (informal, small-scale chicken-slaughterhouses that sell directly to consumers) are still operating, which is the case in many other LMICs. These informal slaughtering facilities are characterized by poor hygiene, inadequate facilities, lack of cold, clean water and inspection during processing. As a result, they are likely to pose a higher risk for consumers (Carron et al., 2017; Cook et al., 2017). Data on *Campylobacter* infection in poultry in Jordan are available. A study carried out in 2012 found *Campylobacter jejuni* in around 40% of the studied broiler farms (Osaili et al., 2012). Al-Natour et al., (2018) investigate the occurrence of *Campylobacter* in 35-layer farms in Northern Jordan and found *C. jejuni* in 40% of chicken cloacae. More recently, a longitudinal study on a single semi-commercial poultry farm, suggests that there may be differences in the transmission dynamics of *Campylobacter* in this type of farms (which are an important source of poultry for the Jordanian population) and those observed in poultry farms in high-income countries, with potentially an earlier introduction of the pathogen into the flock, but slower within-flock transmission (Neves et al., 2019). Data on

the presence of *Campylobacter* in poultry products in Jordan are limited, but evidence from other countries strongly suggests that *Campylobacter* is likely to be present in retail poultry in Jordan. A review conducted in 2009 suggests that in most countries, in which studies have been conducted, a majority of poultry products on retail are contaminated with *Campylobacter* (Suzuki and Yamamoto, 2009). According to the European Food Safety Agency (EFSA) in 2011, in Europe 8 out of 10 chickens were contaminated with *Campylobacter* sp. (EFSA, 2011). The frequency of *Campylobacter* in frozen samples is not common and freezing is suggested as a way for decontaminating slaughtered birds (Ilida and Faridah, 2012),

Macrolides, such as erythromycin and azithromycin, are the treatment of choice for *Campylobacter*, alternatively, fluoroquinolones (ciprofloxacin) and tetracycline can also be used (Osaili and Alaboudi, 2017; Siddiqui et al., 2015). In severe cases such as bacteremia, aminoglycosides (gentamicin) are also used (Alfredson and Korolik, 2007; Corcoran et al., 2006; Kurinčič et al., 2007). Nevertheless, *Campylobacter* resistant strains have increased, probably as a result of the increased use and misuse of antibiotics in poultry farms (Silva et al., 2011), with strains being particularly resistant to chloramphenicol, tetracycline, macrolides, and fluoroquinolones (Cody et al., 2010; EFSA, 2011; Silva et al., 2011).

Currently, Multilocus Sequence Typing (MLST) is the leading and most discriminative method for genotyping (Duarte et al., 2016). It implies the use of housekeeping genes (6–7 genes), which may represent the genome for MLST (Dingle et al., 2001). Relying on the fact that the proteins, which encode these housekeeping genes, evolve slowly, MLST will provide data for accurate phylogenic estimation, typing and strain relatedness (Maiden, 2006). Therefore, the aims of the study were to determine i) the occurrence of *C. jejuni* and *C. coli* in chickens at

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informal butcher shops (fresh), formal poultry slaughterhouses (refrigerated) and retail market (frozen) and ii) the antimicrobial resistance, genotypes and relatedness of the isolates.

2. Materials and methods

2.1. Sample collection

The following formula (Krouse-Wood et al., 2006) was used to determine sample size:

$n = (1.96)^2 PQ/d^2$, whereas:

n = sample size required.

1.96 = z value at α-error = 0.05.

P = prevalence of the disease.

Q = 1 – P.

d = tolerated margin of error.

The reported prevalence rate from previous local studies for chickens was 34.4%, Accordingly the number of samples was determined as:

$$n = ((1.96)^2 \text{ X } 0.344 \text{ X } (1-0.344))/(0.07)^2$$

$$n = 177.$$

Over the period January to May 2017, 177 fresh, refrigerated (not more than one-day old) and frozen chicken samples were obtained. Fresh (59 samples) and refrigerated (59 samples) whole chickens were obtained on a bi-weekly basis from 5 butcher shops and 5 formal slaughterhouses, respectively; 11-12 samples were taken from each site. Imported frozen whole chickens (3 – 6 months from the production date) were also obtained from 5 different local retail market (59 samples). The collected samples were kept in a sterile icebox and taken to the laboratory within

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2-3 h for processing. The selection of the 15 sampling establishments/site and of whole chickens within each was not done probabilistically. In the selection of sampling sites, we aimed to capture variation regarding practices and sources of chickens while limiting the collection area to the surroundings of the city of Irbid (Irbid governorate) in Northern Jordan. A total of 177 was considered feasible and likely to generate enough chicken isolates for comparison.

2.2. Isolation of Campylobacter

In this study, reference strains *C. jejuni* ATCC 33291 and *C. coli* ATCC 43478 (Microbiologics®, Codex, France) were used as positive controls. The ISO 10272-1:2006(E) Horizontal method for detection and enumeration of *Campylobacter* spp. was used with slight modifications. Preston agar (Oxoid, UK) showed better *Campylobacter* spp. recoveries from skin chicken samples and thus was used instead of modified Charcoal Cefoperazone Deoxycholate agar (Chon et al., 2012).

Fifty-gram skin samples from fresh, refrigerated and in-refrigerator thawed chicken samples were taken from the neck, back and wings. The skin of each sample was cut into small pieces and mixed with Bolton broth (Oxoid) in sterile Stomacher bag. Homogenization was performed in Stomacher (Seward, UK) for 2 min at 240 rpm

Subsequently, 10 ml of the chicken samples were transferred into sterile tubes, capped loosely and incubated in a microaerobic atmosphere using Campygen bags (gas generating kits) (Oxoid) at 42°C for 48 h. After incubation, a loopful from each tube was streaked on Preston agar (Oxoid). The plates were set aside to dry in biosafety cabinet and then incubated in a microaerobic atmosphere using Campygen bags at 42°C for 48 h. Plates were then checked for growth and typical *Campylobacter* colonies were chosen.

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Selected colonies from plates, with growth compatible with *Campylobacter* morphology, were re-streaked on Colombia blood agar (Oxoid) and incubated at 42°C for 48 h in a microaerobic atmosphere for further investigation. A loopful of bacteria was taken from plates with heavy growth and re-streaked on Preston agar to check for typical *Campylobacter* colonies.

2.3. Biochemical identification of Campylobacter

Fresh cultures of suspected *Campylobacter* isolates were identified by biochemical means including Oxidase test, Catalase test, Hanging drop motility, Gram Staining, DrySpot Agglutination test and Hipurate hydrolysis test. Furthermore, growth at 25°C microaerobically and at 42°C aerobically was also conducted as recommended by the ISO 10272-1:2006(E) method. Along with each testing session, control strains were tested for comparison.

2.4. Molecular confirmation

DNA extraction and multiplex polymerase chain reaction (mPCR) were performed according to the method described by Nayak et al., (2005). Primers used in the mPCR are available in supplementary materials.

2.5. Antimicrobial susceptibility testing

The European Committee on Antimicrobial Susceptibility Testing disk diffusion method for *C. jejuni* and *C. coli* (EUCAST, 2017) was followed in order to determine the antibiotic susceptibility of the isolates. *C. jejuni* ATCC 33291 was used as control strain. Each isolate was enriched in Mueller-Hinton broth (Oxoid), supplemented with sodium pyruvate, sodium metabisulphite and ferrous sulphate (Oxoid). The inoculum was allowed to reach the turbidity of

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McFarland tube No. 0.5 (absorbance at 600nm was 0.063). When the inoculum reached the desired turbidity, a 0.1 ml aliquot was spread on the surface of Mueller-Hinton agar plate, (Oxoid) supplemented with 5% lysed horse blood (Oxoid). The plates were allowed to dry and the antimicrobial disks were distributed over the plates and incubated at 42°C for up to 48 h. Later, the diameters of the inhibition zone around the disks were measured using a digital caliper. Both *C. jejuni* and *C. coli* were tested for the following antimicrobials: cefoxitin, imipenem, amoxicillin, ciprofloxacin, colistin, ampicillin, aztreonam, cefepime, gentamicin (Oxoid). Interpretation of the resistance/susceptibility of the isolates was done according to the break points proposed by the EUCAST (2017). For antimicrobial breakpoints that are not available in the EUCAST, the Comite De L'antibiogramme De La Societe Francaise De Microbiologie (EUCAST and CA-SFM, 2017) data for *E. coli* were used.

A dendrogram was constructed using MS excel (Microsoft, USA) and the R project which grouped the isolates according to their antimicrobial resistance and susceptibility.

2.6. Multilocus sequence typing

For MLST, along with the housekeeping genes, the purification protocol and the PCR for both *C. jejuni* and *C. coli* were performed according to the method described by Dingle et al. (2001 and 2005). The primers of the housekeeping genes are available in the supplementary materials. The Sanger method was applied on each gene to obtain a sequence of ~500 bp, and the sequences were checked for errors using BioEdit Sequence Alignment Editor (Hall, 1999). Assigning of allele numbers, sequence types (STs) and clonal complexes were conducted by comparison of the data available on the *Campylobacter* MLST library

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(http://pubmlst.org/*Campylobacter*/) (Maiden, 2006), and the trees were constructed using PHYLOViZ online.

2.7. Statistical analysis

The hypothesis, that the proportion of samples in which *Campylobacter* was isolated and antibiotic resistance of the isolates differed between chicken samples from formal slaughterhouses vs. samples from informal butcher shops, was tested by means of the Chi-squared test of association carried out in SPSS® version 25 (IBM, USA). An association was deemed significant when $P<0.05$.

3. Results

3.1. Occurrence of Campylobacter in chicken

Out of 177 chicken samples 64 samples (36.2%), had morphology compatible with that of *Campylobacter* reference strains. Biochemical tests identified *C. coli* in 40 samples and *C. jejuni* in 18. Final confirmation of the isolates was carried out using PCR. The results confirmed that 36 of the isolates were *C. coli* and the remaining 20 isolates were *C. jejuni.* The frequency of *Campylobacter* spp. among the studied samples of local chicken from butcher shops (fresh), local chicken from formal abattoirs (refrigerated) and imported chicken (frozen) was 31.6% (table 1). Overall, *Campylobacter* was isolated in 56 samples of locally produced chickens (47.5%): 21 of samples from formal slaughterhouses (35.6%) and 35 of samples from butcher shops (59.3%), with the difference being statistically significant ($P=0.009$) by Chi-squared test of association). None of imported chicken, (frozen) samples, was found to be contaminated with *Campylobacter* spp.

The proportion of samples in which *C. coli* was isolated was 42.4% for samples obtained from butcher houses and 18.6% for samples obtained from formal slaughterhouses. For *C. jejuni*, the proportions were 17.0% for both butcher houses and formal slaughterhouses.

3.2. Antimicrobial resistance of Campylobacter

A total of 32 chosen *Campylobacter* spp. isolates (13 *C. jejuni*, 19 *C. coli*) were tested for 9 common antibiotics. All isolates, except the reference strain, showed resistance to the treatment of choice (ciprofloxacin), however all samples were susceptible to imipenem (Table 2). High resistance of *C. jejuni* was observed toward cefoxitin, amoxicillin, ciprofloxacin ampicillin, aztreonam, and cefepime, whereas for *C. coli*, high resistance to cefoxitin, ciprofloxacin, ampicillin, aztreonam, and cefepime was found. Low resistance occurred for *C. jejuni* toward imipenem, colistin, and gentamicin whereas for *C. coli* low resistance was found toward imipenem, amoxicillin, colistin, cefepime, and gentamicin. Both species shared susceptibility for imipenem, colistin and gentamicin and resistance for cefepime, ampicillin, aztreonam, ciprofloxacin, ampicillin and cefoxitin. All the isolates revealed multi-antimicrobial resistance toward five or more antimicrobials. No significant differences were found in the susceptibility patterns of the isolates between fresh (butcher shop) and chilled (slaughterhouse) chicken samples.

The dendrogram, which grouped the isolates according to their antimicrobial resistance and susceptibility (Figure 1), revealed that *C. jejuni* and *C. coli* isolates could be grouped in a total of 7 and 10 groups, respectively.

3.3. Genotypes of Campylobacter

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A total of 21 isolates selected from different chicken samples were genotyped. They were chosen according to the groups generated from the dendrogram. Ten *C. coli* isolates of the ST – 828 complex were genotyped. Their sequence types were ST – 902 (4 isolates from group 2), ST – 1595 (2 isolates from group 4) and ST – 830 (4 isolates from group 9) (Table 3). Eleven *C. jejuni* isolates of the 5 complexes (CC – 52, CC – 206, CC – 353, CC – 354 and CC – 464) were genotyped. Their sequence types were ST – 2100, ST – 2282, ST – 2337, ST – 1038, ST – 2813 and ST –9214 (Table 3). Moreover, two phylogenic trees were constructed upon similarities and differences in allelic profiles using PHYLOViZ online (https://online.phyloviz.net). Figure 2 and 3 show the phylogenic tree for both *C. jejuni* and *C. coli* isolates. In figure 2, three sequence types (ST-2337, ST-9214 and ST-2813) are central genotypes which share similarities with other isolates, ST-2337 shared similar allelic number with ST-2282 at *glt* gene, with ST-1038 at *asp*, *glt*, *gly* and *unc* genes and with ST-2813 at *glt* and *gly* genes. ST-9214 shared similar allelic number with st-2100 at *glt* gene and with ST-2813 at *asp*, *gly*, *pgm* and *unc* genes. In figure 3, similarities among *C. coli* sequence types occurred at *asp*, *glt*, *gly*, *pgm* and *unc* genes where similar allelic numbers were identified.

Genotyping data of the current study can be found at pubmlst database website (https://pubmlst.org/bigsdb?db=pubmlst_campylobacter_isolates&page=query), accession IDs for *C. coli* isolates are from 105789 to 105798 and for *C. jejuni* isolates are from 105799 to 105809.

4. Discussion

Chicken meat has been identified as the main source of human *Campylobacter* infection and studies aiming at attributing the source of human infection or the relatedness of isolates are conducted (Nadeau et al., 2002; Ravel et al., 2017). The occurrence of *Campylobacter* among

chickens sold in Jordan included in this study (31.6% overall among fresh, refrigerated and frozen) is comparable to results from different countries such as Italy 34% (Stella et al., 2017), China 45% (Zhu et al., 2017), Pakistan 29% (Nisar et al., 2017), Brazil 17% (da Silva et al., 2016), Germany 38% and Hungary 24% (Skarp et al., 2016). Such a relatively high frequency of occurrence in Jordan is not unexpected, as chickens are traditionally raised without strict biosecurity measures (Al-Natour et al., 2016) and the low levels of hygiene practiced at the local slaughterhouses increase the risk of cross-contamination (Osaili et al., 2012).

Among broilers produced locally, in formally approved slaughterhouses and informal small-scale butcher shops, indicated that the occurrence of *Campylobacter* was significantly higher in the later (35.6% vs. 59.3%), suggesting that stricter hygienic measures and cooled facilities applied in approved slaughterhouses may play an important role in controlling of *Campylobacter* cross-contamination at slaughter (Nisar et al., 2017). However, it cannot be ruled out that the differences are at least in part due to different levels of contamination at origin, as different types of farms may supply the two types of slaughtering facilities. Zero prevalence of *Campylobacter* among imported frozen broilers has been reported in previous studies in countries such as Belarus, Russia or Malaysia (Suzuki and Yamamoto, 2009; Ilida, and Faridah, 2012). The damage induced by freezing to the outer membrane of *Campylobacter* cells makes isolation of cells difficult, which may result in false negatve results (Ilida and Faridah, 2012). The most prevalent *Campylobacter* species in poultry are *C. jejuni* and *C. coli* (da Silva et al., 2016; Nisar et al., 2017; Skarp et al., 2016; Zhu et al., 2017). A higher frequency of *C. coli* 64% (36/56) than *C. jejuni* 35% (20/56) has been observed in the current study. Similar results have been obtained by Kurinčič (Kurinčič et al., 2007) and Lynch (Lynch et al., 2011) where they reported that the use of Preston media and broth for isolation favored the recovery of *C. coli*.

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Other possible explanation is that in samples with high levels of contamination, the numbers of *C. coli* could dominate. This was the case for samples from butcher shops (*C. coli: C. jejuni* 2.5:1), whereas, the ratio was almost 1:1 among the formal slaughterhouse isolates.

A key driver for the development of antimicrobial resistance in foodborne pathogens is the misuse of veterinary drugs (Hoszowski and Wasyl, 2005). The use of antibiotics in livestock and poultry poses a public health risk as some antibiotics are used in both, humans and animals (Luangtongkum et al., 2009). In Jordan, there are no strict regulations on the use of antibiotics in animals. The treatments of choice for *Campylobacter* spp. infection are macrolides, such as erythromycin and fluoroquinolones (ciprofloxacin) (Silva et al., 2011). All tested isolates (*C. jejuni* and *C. coli*) were resistance to ciprofloxacin which is in agreement with results from Algeria, Poland and Latvia and Estonia where 83.7% (Messad et al., 2014), 66.3% (Andrzejewska et al., 2015) and 60% (Kovaļenko et al., 2014; Roasto et al., 2007) of the isolates showed resistance to this antibiotic. A recent study in Jordan also found that all *Campylobacter* spp. patients' isolates were resistant to erythromycine (Osaili et al., 2012).

Lower resistance to gentamicin (15.4% *C. jejuni* and 36.8% *C. coli*) is in agreement with the results of studies from Pakistan 25.6% (Nisar et al., 2017), Ireland 6.3% (Wilson, 2003), and Poland 5%, (Wieczorek and Osek, 2013).

All samples were susceptible to imipenem which is expensive in comparison with other drugs, and with no significant use in animals. Similar results were observed in human isolates from Finland and Kuwait (Albert, 2013; Hakanen et al., 2003) and from Jordan (Jaradat, 2015) where *Campylobacter* human isolates showed 100% susceptibility towards imipenem. This may suggest the possibility of using imipenem as an alternative to erythromycin or ciprofloxacin in the management of human infection.

Most of the isolates were resistant against 5 or less antibiotics (62.5%) whereas the remaining (37.5%) resisted 6 antibiotics or more; all isolates were considered as multi-drug resistant. High multidrug resistance rates among *Campylobacter* have been shown in previous studies in Asia, Africa, Europe and America. A previous study in Jordan (Osaili et al., 2012) reported 55% (n=21) of 38 *Campylobacter* isolates were multidrug resistant. Al-Natour et al. (2016) also reported 100% (n=92) resistance towards ciprofloxacin and 84% resistance towards gentamicin on isolates from layer farms in Jordan and attributed such high resistance to the longer life span of layer hens compared to broilers.

All identified *C. coli* genotypes were of ST-828 complex and the sequence groups characterized were ST-902, ST-1595 and ST-830. In Finland, ST-828 complex was almost the only CC in chicken, cattle, turkey, wild birds and environmental water samples (Sheppard et al., 2009). The results of *C. coli* genotyping indicate a low diversity, which might be related to imprecise limited source of *C. coli* in Jordan. This might also prove that the high numbers of *C. coli* reported in this study came from cross-contamination rather than an actual chicken source.

Among *C. jejuni* isolates, 6 sequence types were detected and belonged to 5 clonal complexes. The ST-2100 which belonged to the ST-52 complex was reported in poultry from Germany (Rosner et al., 2017). The ST-9214 and ST-2813 shared four identical allels, both belonged to the ST-464 complex and both sequence types were reported only in humans from USA, UK and Luxemburg where 60% of human cases were attributed to poultry (Mossong et al., 2016; Sheppard et al., 2009). The results of *C. jejuni* genotyping indicate a higher diversity than *C. coli*, this might be due to different sources of contamination like in farm or in slaughterhouse.

Overall, the high frequency of occurrence of *Campylobacter* in poultry, the high resistance towards many antimicrobials and the shared similarities among isolates support a

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potentially important public health burden associated with *Campylobacter* from poultry sources. Hygienic measures at slaughterhouses are likely to play an important role in reducing cross-contamination. It is likely that unregulated use of antibiotics has led to high levels of resistance in Jordan, where the higher diversity of *C. jejuni* compared to *C. coli,* may be explained by the fact that not many sources are present for *C. coli.*

Acknowledgment

This work was part of the OIE Veterinary Education Twinning Programme between The Royal Veterinary College and the Jordan University of Science and Technology (grant number AD/VW/2017/365), funded by the UK Biosecurity Programme. The study was partly funded by Jordan University Science and Technology.

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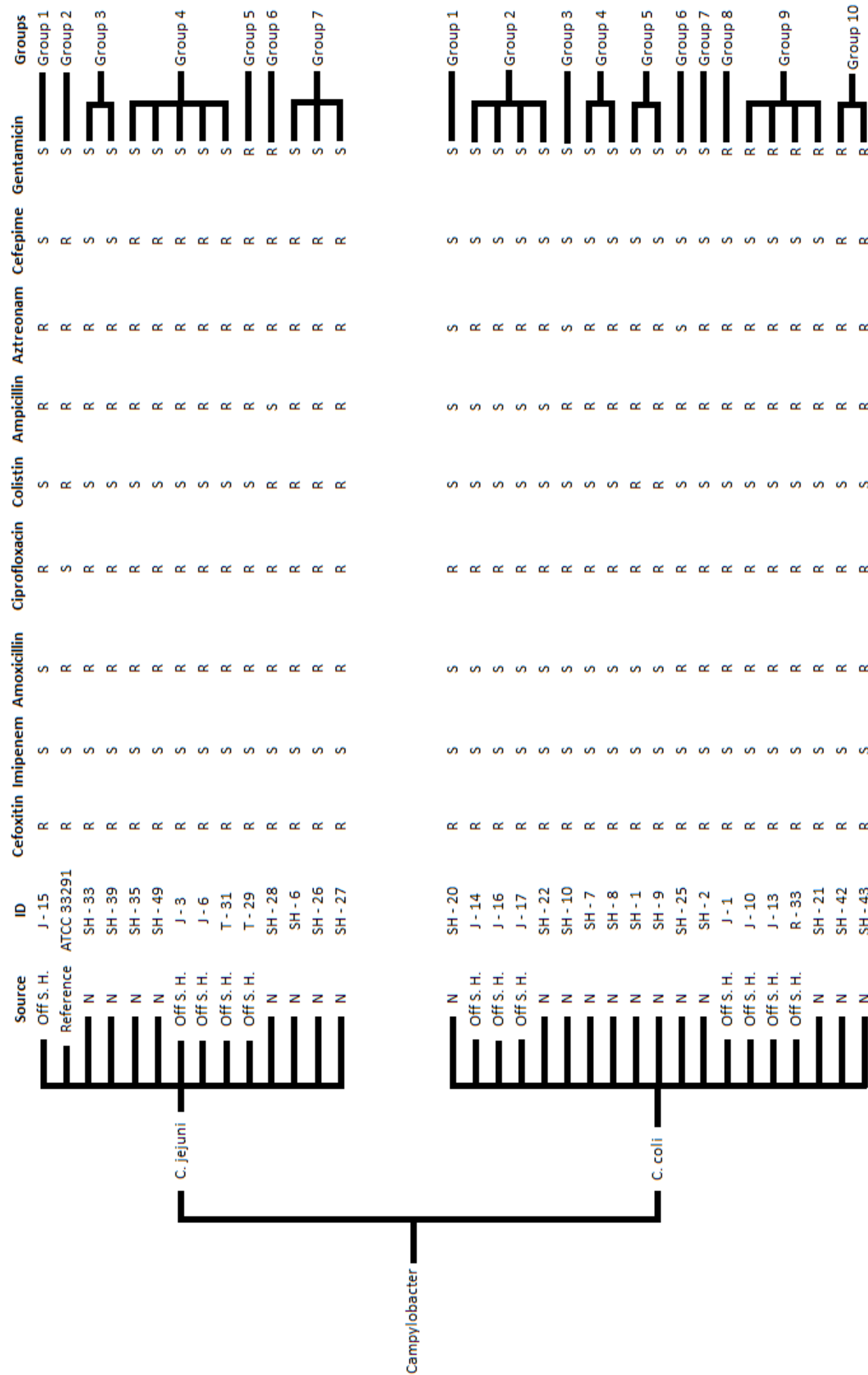

Species	Source	ID	Cefoxitin	Imipenem	Amoxicillin	Ciprofloxacin	Colistin	Ampicillin	Aztreonam	Cefepime	Gentamicin	Groups
C. jejuni	Off S. H.	J - 15	R	S	S	R	S	R	R	S	S	Group 1
C. jejuni	Reference	ATCC 33291	R	S	R	S	R	R	R	R	S	Group 2
C. jejuni	N	SH - 33	R	S	R	R	S	R	R	S	S	Group 3
C. jejuni	N	SH - 39	R	S	R	R	S	R	R	S	S	Group 3
C. jejuni	N	SH - 35	R	S	R	R	S	R	R	R	S	Group 4
C. jejuni	N	SH - 49	R	S	R	R	S	R	R	R	S	Group 4
C. jejuni	Off S. H.	J - 3	R	S	R	R	S	R	R	R	S	Group 4
C. jejuni	Off S. H.	J - 6	R	S	R	R	S	R	R	R	S	Group 4
C. jejuni	Off S. H.	T - 31	R	S	R	R	S	R	R	R	S	Group 4
C. jejuni	Off S. H.	T - 29	R	S	R	R	S	R	R	R	R	Group 5
C. jejuni	N	SH - 28	R	S	R	R	R	S	R	R	R	Group 6
C. jejuni	N	SH - 6	R	S	R	R	R	R	R	R	S	Group 7
C. jejuni	N	SH - 26	R	S	R	R	R	R	R	R	S	Group 7
C. jejuni	N	SH - 27	R	S	R	R	R	R	R	R	S	Group 7
C. coli	N	SH - 20	R	S	S	R	S	S	S	S	S	Group 1
C. coli	Off S. H.	J - 14	R	S	S	R	S	S	R	S	S	Group 2
C. coli	Off S. H.	J - 16	R	S	S	R	S	S	R	S	S	Group 2
C. coli	Off S. H.	J - 17	R	S	S	R	S	S	R	S	S	Group 2
C. coli	N	SH - 22	R	S	S	R	S	S	R	S	S	Group 2
C. coli	N	SH - 10	R	S	S	R	S	R	S	S	S	Group 3
C. coli	N	SH - 7	R	S	S	R	S	R	R	S	S	Group 4
C. coli	N	SH - 8	R	S	S	R	S	R	R	S	S	Group 4
C. coli	N	SH - 1	R	S	S	R	R	R	R	S	S	Group 5
C. coli	N	SH - 9	R	S	S	R	R	R	R	S	S	Group 5
C. coli	N	SH - 25	R	S	R	R	S	R	S	S	S	Group 6
C. coli	N	SH - 2	R	S	R	R	S	R	R	S	S	Group 7
C. coli	Off S. H.	J - 1	R	S	R	R	S	R	R	S	R	Group 8
C. coli	Off S. H.	J - 10	R	S	R	R	S	R	R	S	R	Group 9
C. coli	Off S. H.	J - 13	R	S	R	R	S	R	R	S	R	Group 9
C. coli	Off S. H.	R - 33	R	S	R	R	S	R	R	S	R	Group 9
C. coli	N	SH - 21	R	S	R	R	S	R	R	S	R	Group 9
C. coli	N	SH - 42	R	S	R	R	S	R	R	R	R	Group 10
C. coli	N	SH - 43	R	S	R	R	S	R	R	R	R	Group 10

Fig. 1. C. *jejuni* and *C. coli* isolate groups according to the antibiotic resistance and susceptibility for nine antibiotics. (S.H.: slaughterhouse; N: butcher shop; J, T, R, name of abattoirs; S, susceptible; R, resistant).

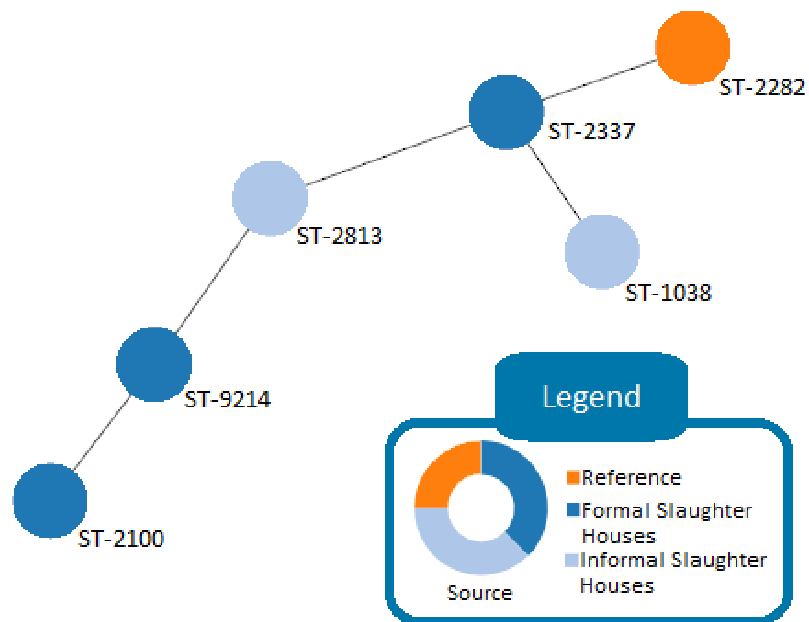

Fig. 2. Phylogenic tree by sequence typing of C. *jejuni* isolates. (ST, Sequence Type).

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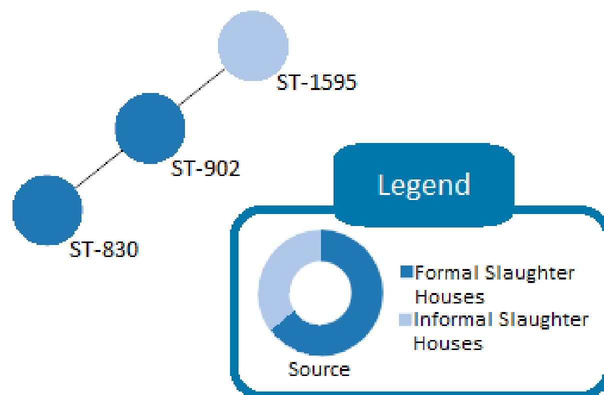

Fig. 3. Phylogenic tree by sequence typing of *C. coli* isolates (ST, Sequence Type).

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Table 1. Number and proportion of chicken (n=177) samples in which *C. coli* and *C. jejuni* were confirmed by PCR.

***Campylobacter* sp.**	**Chicken samples**		
	Fresh samples from butcher shops (n=59)	Refrigerated samples from formal slaughterhouses (n=59)	Frozen sample from retail market (n=59)
C. coli	25 (42.4%)	11 (18.6%)	0
C. jejuni	10 (17.0%)	10 (17.0%)	0
Total	35 (59.3%)	21 (35.6%)	0

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Table 2. Antibiotic resistance/susceptibility of *Campylobacter* isolates from chicken (n=32) (19 *C. coli*, 13 *C. jejuni*)

Antibiotic	*Break Points*		*C. jejuni*		*Break Points*		*C. coli*	
	S≥	**R<**	**R (%)**	**S (%)**	**S≥**	**R<**	**R (%)**	**S (%)**
Cefoxitin	19	15	100	0	19	15	100	0
Imipenem	22	16	0	100	22	16	0	100
Amoxicillin	19	14	81	19	19	14	47	53
Ciprofloxacin	26	26	100	0	26	26	100	0
Colistin	15	15	43	57	15	15	10	90
Ampicillin	19	14	81	19	19	14	73	27
Aztreonam	26	21	100	0	26	21	84	16
Cefepime	27	21	68	32	27	21	10	90
Gentamicin	17	17	12	88	17	17	36	64

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Table 3. Genotyping results of *Campylobacter* spp. of chicken isolates (n=21).

Species	Sequence Type	Source	Total Number	Clonal Complex	Accession ID
C. coli	ST-902	Poultry	4	ST-828 Complex	105789, 105790, 105791, 105792
	ST-1595	Poultry	2	ST-828 Complex	105793, 105794
	ST-830	Poultry	4	ST-828 Complex	105795, 105796, 105797, 105798
C. jejuni	ST-2100	Poultry	1	ST-52 Complex	105799
	ST-2282	Poultry	1	ST-206 Complex	105800
	ST-2337	Poultry	5	ST-353 Complex	105803, 105804, 105805, 105806, 105807
	ST-1038	Poultry	1	ST-354 Complex	105809
	ST-2813	Poultry	2	ST-464 Complex	105801, 105802
	ST-2914	Poultry	1	ST-464 Complex	105808

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Highlights

- Prevalence rate of *Campylobacter* in chickens was 31.6%
- *C. coli* was isolated more from chickens from informal than formal slaughterhouses
- All tested isolates were multidrug resistant to 5 or more antimicrobials
- Chicken isolates shared similarities at phenotyping and genetic levels

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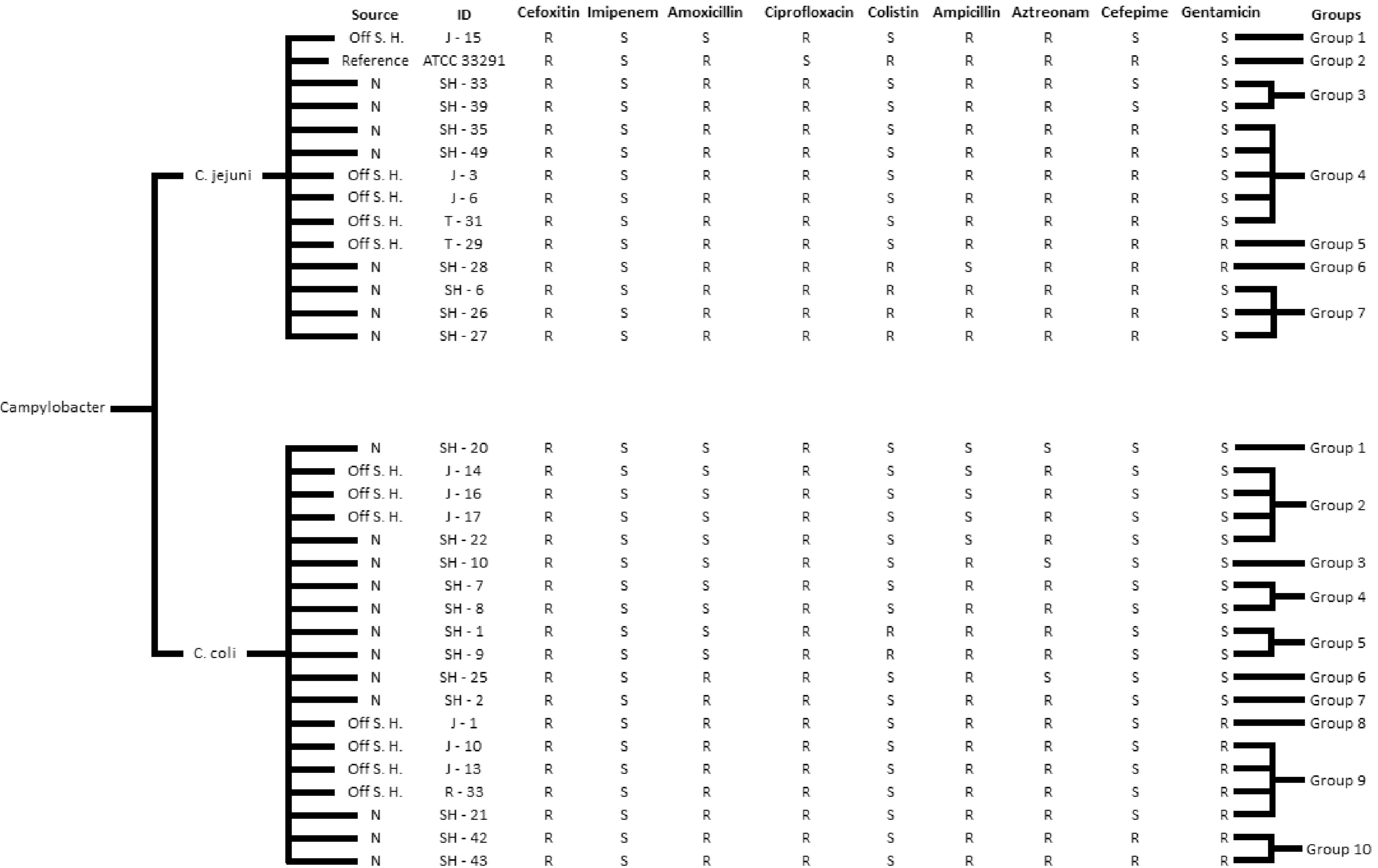

Figure 1

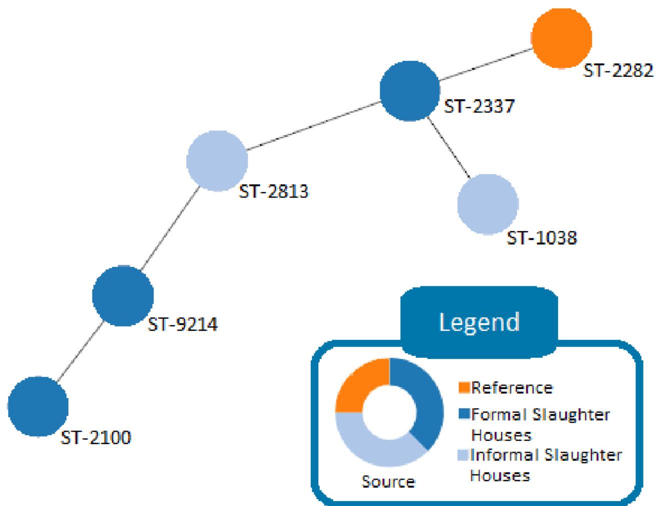

Figure 2

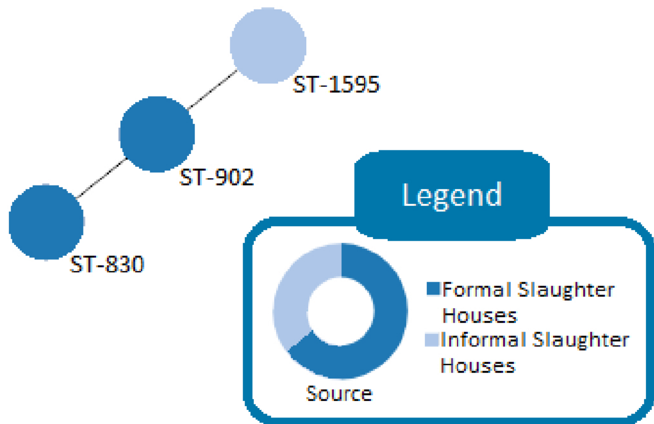

Figure 3