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Original Article

Virulence, MLST analysis, and antimicrobial resistance of *Campylobacter coli* isolated from broiler chickens in Tamil Nadu, India

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Abstract

Background: *Campylobacter* species are the zoonotic bacteria and the most common cause of foodborne gastroenteritis around the world. The link between human campylobacteriosis and infected poultry consumption has been well established. **Aims:** In this study, we aimed to isolate *Campylobacter* spp. from chicken and characterize them with molecular methods. **Methods:** Totally, 241 chicken caecal mucosal scrapings were collected from five districts of Tamil Nadu. Bacterial isolation was done by plating on blood-free *Campylobacter* selective medium with supplements. *Campylobacter* species were identified by multiplex PCR and *Campylobacter coli* isolates were tested for 11 virulence genes by PCR. *C. coli* isolates were typed based on seven housekeeping genes multilocus sequence typing (MLST) scheme. The antimicrobial susceptibility was determined by a microdilution resazurin assay. **Results:** The prevalence of *C. coli* and *C. jejuni* were 14.94% (36/241), and 3.32% (8/241), respectively. The virulence genes *flaA*, *flaB*, *cadF*, *cdtA*, *cdtB*, *cdtC*, *ciaB*, and *ceuE* were present in all 36 *C. coli* isolates, *pldA* and *racR* genes were present in 58.33% (21/36), and 16.67% (6/36) of the isolates, respectively, and *dnaJ* was present in only one isolate. Two novel sequence types (ST-10872, ST-11031) were found in this study. Though different STs were identified, all the STs belonged to the same clonal complex of ST-828. All 14 *C. coli* isolates showed 100% resistance to nalidixic acid, and higher resistance to tetracycline (92.8%), erythromycin (71.4%), clindamycin (71.4%), and azithromycin (64.2%) was noticed. All *C. coli* isolates were sensitive to chloramphenicol, and higher sensitivity to ciprofloxacin (78.5%), and gentamicin (71.4%) was observed. **Conclusion:** The present study demonstrated that *C. coli* is more prevalent in broilers than *C. jejuni* in Tamil Nadu. The presence of *C. jejuni* and *C. coli* in chicken caecal samples from the slaughterhouse are indicative of the possibility of public health hazards.

Key words: Antimicrobial resistance, *Campylobacter coli*, Multilocus sequence typing, Multiplex PCR, Virulence genes

Introduction

India is one of the world's countries with the largest poultry population, and in this country, poultry meat consumption is commonly high due to Indian culture (Khan *et al*., 2018). The poultry population in Tamil Nadu had a tremendous growth rate and reached a total population of 120.8 million in 2019, the highest poultry population in India (Livestock Census of India, 2019). There is a risk of transmission of diseases from poultry in such an intensive state. Campylobacteriosis is a zoonotic disease that is predominantly spread from poultry and poultry products. The prevalence of *Campylobacter* spp. in chicken and its products were reported to be 4.9% to 100% globally (Kaakoush *et al*., 2015).

All the domestic poultry species like chickens, turkeys, ducks, and geese harbours *Campylobacter* spp., but the highest colonisation and prevalence was seen in chickens (Byrd *et al*., 2001). Despite the highest colonisation rate, there were no clinical symptoms or reduced life span in chickens (Sahin *et al*., 2001). Consumption of poultry meat was incriminated for 20% to 30% of campylobacteriosis cases in the European Union (Skarp *et al*., 2016). There are currently 17 species, and six subspecies in the *Campylobacter* genus. *C. jeuni* and *C. coli* are the most common species causing gastrointestinal infections in humans, but *C. lari* and *C. upsaliensis* are rarely associated with human infection (Skarp *et al*., 2016). In recent studies, *C. coli* was found to be more prevalent than *C. jejuni* in most countries (Walker *et al*., 2019; Wieczorek *et al*., 2020). Because of the phenotypic similarities between *Campylobacter* spp., identification of the individual

species is difficult. Various PCR-based detection methods have been proposed. Multiplex PCR and LAMP assay could help in the fast and effective diagnosis of *Campylobacter* spp. (Wang *et al*., 2002; Toplak *et al*., 2012; Romero *et al*., 2018).

The *Campylobacter* genus has complex multifactorial systems that include motility, chemotaxis, adherence, invasion, antioxidant resistance, heat shock, and the ability to enter the viable but not cultivable (VBNC) state (to replicate in chickens), survive during food processing, and cause virulence in humans (Bolton *et al*., 2015). The virulence capacity of *Campylobacter* spp. was found to be increased in humans when passed through the poultry (Wysok *et al*., 2020).

Antimicrobial resistance is a worldwide threat. Antibiotics such as fluoroquinolones, macrolides, and tetracycline are widely used to treat campylobacteriosis. However, resistance to these antibiotics develops quickly (Engberg *et al*., 2001). Campylobacter's resistance to fluoroquinolones had been identified even in the absence of their use (Caffrey *et al*., 2021).

Campylobacteriosis is a sporadic disease, and the detection of the bacterial sources is epidemiologically difficult. Multilocus sequence typing (MLST) of *C. jejuni/coli* assists in the recognition of its sequence types and clonal complex associated with the source of isolation. MLST could aid in effective public-health measures to control campylobacteriosis (Vasiliki *et al*., 2013). There is no previous study on the prevalence of *C. jejuni/coli* sequence types or the most common clonal complexes in India. Hence, this research aimed to detect virulence factors, type the *C. coli* isolates from chickens, and determine the minimum inhibitory concentration (MIC) of *C. coli* isolates against various antimicrobials.

Materials and Methods

Sample collection and *Campylobacter* spp. isolation

A total of 241 chicken intestine samples were collected from five different districts of Tamil Nadu. Among the 241 samples, 97 intestine samples were collected from five various areas of the Chennai district, and 36 samples each from six separate areas of Namakkal, Krishnagiri, Erode, and Coimbatore districts. Chicken caecal contents were cleared, and the mucosal layer was scraped with the help of a glass slide. Scraped contents of samples from the Chennai district were directly streaked on the sterile blood-free *Campylobacter* broth base (HiMEDIA catalogue #M1318) with 2% Agar agar type 1, *Campylobacter* growth supplement IV (HiMEDIA catalogue #FD042), and CCDA supplement (HiMEDIA catalogue #FD135), then the plates were incubated at 42°C for 48 h in microaerophilic condition. Scraped caecal contents from other districts were transferred into a 1.5 ml sterile microcentrifuge tube containing 1 ml of blood free *Campylobacter* broth with CCDA, and *Campylobacter* growth supplement IV, then transported to the laboratory for *Campylobacter* isolation as mentioned above.

Colonies on the selective media were checked by staining with dilute carbol fuchsin, oxidase, and catalase tests for presumptive identification as *Campylobacter.*

Multiplex PCR

To confirm *Campylobacter* spp. isolates, multiplex PCR was used. Bacterial genomic DNA was extracted by boiling method (Ananda Chitra *et al*., 2015). Briefly, each colony was suspended in nuclease-free water and boiled at 100°C for 10 min. The supernatant was separated by centrifugation at 11,200 × g for 10 min and later used as a template in the amplification reactions. The details of primers are given in Table 1. Multiplex PCR was performed in 20 µL reaction volume containing 100-150 ng of DNA, 0.2 µM of *23S rRNA* primers, 0.5 µM of *C. jejuni* and *C. lari* primers, 1 µM of *C. coli* primers and 2 µM *C. upsaliensis* primers in 2X master mix (Ampliqon, Denmark) with the following conditions: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 30 s and elongation at 72°C for 30 s, and a final elongation of 72°C for 7 min. Amplified PCR products were electrophoresed in 2% agarose gel stained with ethidium bromide, visualized under UV, and documented.

Campylobacter virulence genes and virulence gene profiling

The *Campylobacter* isolates were tested for the presence of 11 virulence genes using PCR. The virulence

Table 1: Multiplex PCR primers along with amplicon size, and references used for identification of *Campylobacter* spp.

S. No.	Gene/species	Primer sequences (5´-3´)	References	Product size bp
1	*23S rRNA*	F-TATACCGGTAAGGAGTGCTGGAG R-ATCAATTAACCTTCGAGCACCG	Wang *et al*. (2002)	650
2	*hipO* *C. jejuni*	F-ACTTCTTTATTGCTTGCTGC R-GCCACAACAAGTAAAGAAGC	Wang *et al*. (2002)	323
3	*glyA* *C. coli*	F-GTAAAACCAAAGCTTATCGTG R-TCCAGCAATGTGTGCAATG	Wang *et al*. (2002)	126
4	*glyA* *C. lari*	F-TAGAGAGATAGCAAAAGAGA R-TACACATAATAATCCCACCC	Wang *et al*. (2002)	251
5	*glyA* *C. upsaliensis*	F-AATTGAAACTCTTGCTATCC R-TCATACATTTTACCCGAGCT	Wang *et al*. (2002)	204

Table 2: Primers along with amplicon size, annealing temperature, and references used for the detection of virulence genes in *Campylobacter coli*

S. No.	Gene	Primer sequence (5´-3´)	References	Annealing Temp. °C	Product size bp
1	*flaA*	F-AATAAAAATGCTGATAAAACAGGTG R-TACCGAACCAATGTCTGCTCTGATT	Datta *et al*. (2003)	53	855
2	*flaB*	F-AAGGATTTAAAATGGGTTTTAGAATAAACACC R-GCTCATCCATAGCTTTATCTGC	Goon *et al*. (2003)	57	260
3	*cadF*	F-TTGAAGGTAATTTAGATATG R-CTAATACCTAAAGTTGAAAC	Konkel *et al*. (1999)	48	400
4	*dnaJ*	F-AAGGCTTTGGCTCATC R-CTTTTTGTTCATCGTT	Datta *et al*. (2003)	41	720
5	*pldA*	F-AAGAGTGAGGCGAAATTCCA R-GCAAGATGGCAGGATTATCA	Zheng *et al*. (2006)	55	384
6	*racR*	F-GATGATCCTGACTTTG R-TCTCCTATTTTTACCC	Datta *et al*. (2003)	45	584
7	*cdtA*	F-TGTCCCACCTGTAATCACTCC R-CTCTTGCATCTCCAAAAGGTCT	Denis *et al*. (2017)	57	245
8	*cdtB*	F-GAGTGGATGTAGGAGCAAATCG R-CGTAGAAGAAGGCGGAACAAC	Denis *et al*. (2017)	57	332
9	*cdtC*	F-AGCTTGGATGAATTAGCAGACT R-TGGCGATACTAGAGTCAGGAAA	Denis *et al*. (2017)	57	403
10	*ciaB*	F-TGCGAGATTTTTCGAGAATG R-TGCCCGCCTTAGAACTTACA	Denis *et al*. (2017)	57	284
11	*CeuE*	F-ATGAAAAAATATTTAGTTTTTGCA R-ATTTTATTATTTGTAGCAGCG	Gonzalez *et al*. (1997)	49	894

genes include those involved in motility (*flaA*, *flaB*), cell adhesion (*cadF*, *dnaJ*, *pldA* and *racR*), invasion (*ciaB*, *ceuE*), and cytotoxin production (*cdtA*, *cdtB*, *cdtC*). The details of the PCR primers are shown in Table 2. For virulence gene profile analysis, binary code 1 was used for gene presence and 0 for gene absence. Hierarchical clustering was carried out using Displayr software (https://app.displayr.com/).

Multilocus sequence typing (MLST)

C. coli isolates were typed based on seven housekeeping genes including, *aspA* (aspatase), *glnA* (glutamine synthetase), *gltA* (citrate synthase), *glyA* (serine hydroxy methyl transferase), *pgm* (phospho glucomutase), *tkt* (transketolase), and *uncA* (ATP synthase alpha subunit). Primers details used for MLST are given in Table 3. PCR was performed in 20 µL reaction volume for each gene of each isolate. The reaction mixture containing 100-150 ng of DNA, and 1 µM of each primer in 2X PrimeStar master mix (Takara Bio Inc., Japan) which contained high fidelity hot-start DNA polymerase. PCR was performed under the thermal conditions of initial denaturation at 98°C for 2 min, followed by 34 cycles of denaturation at 98°C for 10 s, annealing at 53°C for 30 s and elongation at 72°C for 30 s, and a final elongation of 72°C for 5 min. Amplified PCR products were loaded in 1.5% agarose gel stained with ethidium bromide, and electrophoresed, then visualized by a transilluminator. The PCR products were purified by a gel purification kit (Favorgen, catalogue #FAGC 001) according to the manufacturer's instructions. Purified PCR products were sequenced by Sanger's DNA sequencing method in a commercial firm (Eurofins Genomics India Pvt. Ltd.). Forward and reverse sequences for each gene were aligned using the MEGAX program. Allele numbers and profiles were documented by submitting the aligned sequences on the PubMLST website (https://pubmlst.org/organisms/campylobacter-jejunicoli/) (Jolley *et al*., 2018).

Minimum inhibitory concentration (MIC)

The antimicrobial susceptibility was determined by a microdilution resazurin assay. Briefly, the antibiotic powders containing, azithromycin, chloramphenicol, clindamycin, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, and tetracycline were individually diluted in suitable diluents to make 1 mg/ml stock solutions. The colonies were directly transferred from the plate into *Brucella* broth with 10% horse serum, and the suspension was matched to the 0.5 McFarland standard. The antibiotics starting from 100 µg/ml concentration, were diluted at two folds in *Brucella* broth with 10% horse serum in flat bottom sterile cell culture plate, and 100 µL of bacterial suspension was added to all wells. After 24 h incubation at 42°C under microaerophilic conditions, 10 µL (0.2%) of resazurin dye was added to each well, and plates were further incubated at 42°C for 3 h, then observed for the colour change. CLSI breakpoints (2020) were used for determining the MIC of erythromycin, ciprofloxacin, and tetracycline, and NARMS breakpoints (2020) were used for the other tested antimicrobials.

Table 3: Primers along with amplicon size, and references used for MLST amplification and sequencing of *C. coli* isolates

S. No.	Gene	Primer sequence (5´-3´)	References	Product size (bp)
1	*aspA*	F-CAACTTCAAGATGCAGTACC R-ATCTGCTAAAGTATGCATTGC	Dingle *et al*. (2001)	594
2	*glnA*	F-TTCATGGATGGCAACCTATTG R-GCTTTGGCATAAAAGTTGCAG	Dingle *et al*. (2001)	615
3	*gltA*	F-GATGTAGTGCATCTTTTACTC R-AAGCGCTCCAATACCTGCTG	Dingle *et al*. (2001)	528
4	*glyA*	F-TCAAGGCGTTTATGCTGCAC R-CCATCACTTACAAGCTTATAC	Dingle *et al*. (2001)	627
5	*Pgm*	F-TTATAAGGTAGCTCCGACTG R-GTTCCGAATAGCGAAATAACAC	Dingle *et al*. (2001)	649
6	*Tkt*	F-AGGCTTGTGTTTTCAGGCGG R-TGACTTCCTTCAAGCTCTCC	Dingle *et al*. (2001)	581
7	*uncA*	F-AAGCACAGTGGCTCAAGTTG R-CTACTTGCCTCATCCAATCAC	Dingle *et al*. (2001)	614

Results

Prevalence of *Campylobacter* spp. in chicken

Out of 241 samples, 14.94% (36/241), and 3.32% (8/241) were found positive for *C. coli*, and *C. jejuni*, respectively, by multiplex PCR (Fig. 1). None of the samples was positive for C. *lari* and *C. upsaliensis.* Among 36 *C. coli* isolates, 35 were isolated from Chennai district, and one was isolated from Namakkal district of Tamil Nadu.

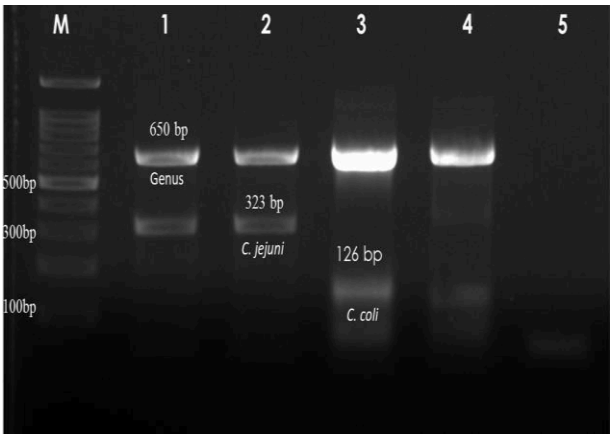

Fig. 1: Agarose gel showing the multiplex PCR products of *Campylobacter* spp., *C. jejuni* and *C. coli*. M: 100 bp DNA marker. Lanes 1-2: *C. jejuni*, Lane 3: *C. coli*, Lane 4: *Campylobacter* spp., and Lane 5: Non-template control

Virulence genes profile

Virulence genes detection (Figs. 2 and 3) in 36 *C. coli* isolates showed that genes involved in motility (*flaA* and *flab*), invasion (*ciaB*, *ceuE*), and cytotoxin production (*cdtA*, *cdtB*, *cdtC*) were present in all the isolates, whereas the prevalence of genes involved in cell adhesion varies in *C. coli* isolates. The *cadF*, *pldA*, and *racR* genes were present in 100% (36/36), 58.33% (21/36), and 16.67% (6/36) of the isolates, respectively. The cell adhesion factor *dnaJ* was found in only one of the isolates (2.78%). A total of four different clusters and one individual virulence profile (VPs) were observed (Fig. 4). Virulence genes profile *flaA-flaB-pldA-cadF-cdtA-cdtB-cdtC-ciaB-ceuE* (VP5) had the maximum number of strains (50%), followed by VP3 with 31% (11/36), and VP2 with 11% (4/36) of the isolates.

Multilocus sequence typing

Among the 36 *C. coli* isolates, five randomly selected *C. coli* isolates were subjected to the MLST sequencing method (Fig. 5). The details of the allele number, sequence type (ST), and clonal complex (CC) are given in Table 4. Allele numbers for *glnA*, *gltA*, and *uncA* were the same for all the five *C. coli* isolates. In contrast, *tkt* gene sequences were highly variable, which was evident by displaying four different alleles within five isolates. In this study, two novel STs were found in the Chennai area, and the newly assigned sequence types were ST-10872 and ST-11031. However, all the STs, including two new ones, belonged to the same clonal complex ST-828.

Antimicrobial resistance of *C. coli* isolates

All the 14 tested *C. coli* isolates were resistant to nalidixic acid (100%). Higher resistance was shown to tetracycline (92.8%), erythromycin (71.4%), and clindamycin (71.4%). For azithromycin, 64.2% of the isolates were resistant. On the contrary, all the *C. coli* isolates (100%) were sensitive to chloramphenicol. For ciprofloxacin, and gentamicin, 78.5%, and 71.4% of the isolates were respectively sensitive. In the present study, all the *C. coli* isolates were resistant to at least one tested antimicrobial. Three isolates exhibited an azithromycin-clindamycin-erythromycin-nalidixic acid-tetracycline antimicrobial resistant pattern. Five isolates were resistant to 5 tested antimicrobials, and three isolates were resistant to six different antimicrobials. One isolate was found to be sensitive to all antimicrobials except for nalidixic acid. Results of antimicrobial sensitive patterns are shown in Table 5.

Discussion

Campylobacter spp. are zoonotic bacteria that cause

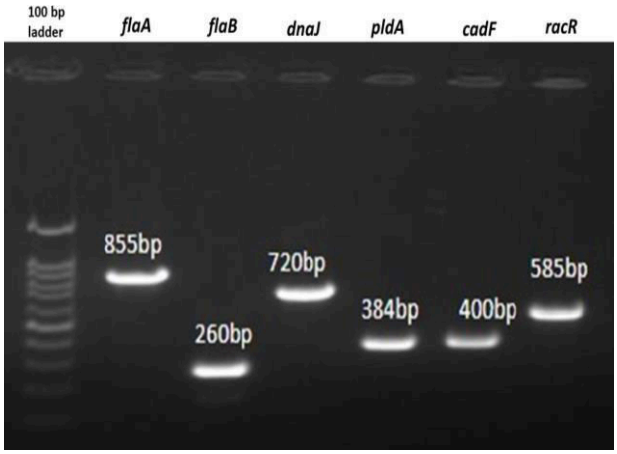

Fig. 2: Agarose gel showing the PCR products of virulence genes *flaA*, *flaB*, *dnaJ*, *pldA*, *cadF*, and *racR* of *C. coli* isolates

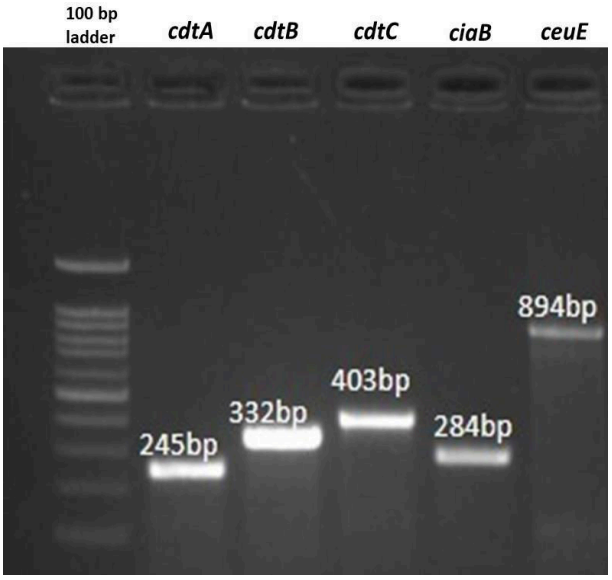

Fig. 3: Agarose gel showing the PCR products of virulence genes *cdtA*, *cdtB*, *cdtC*, *ciaB*, and *ceuE* of *C. coli* isolates

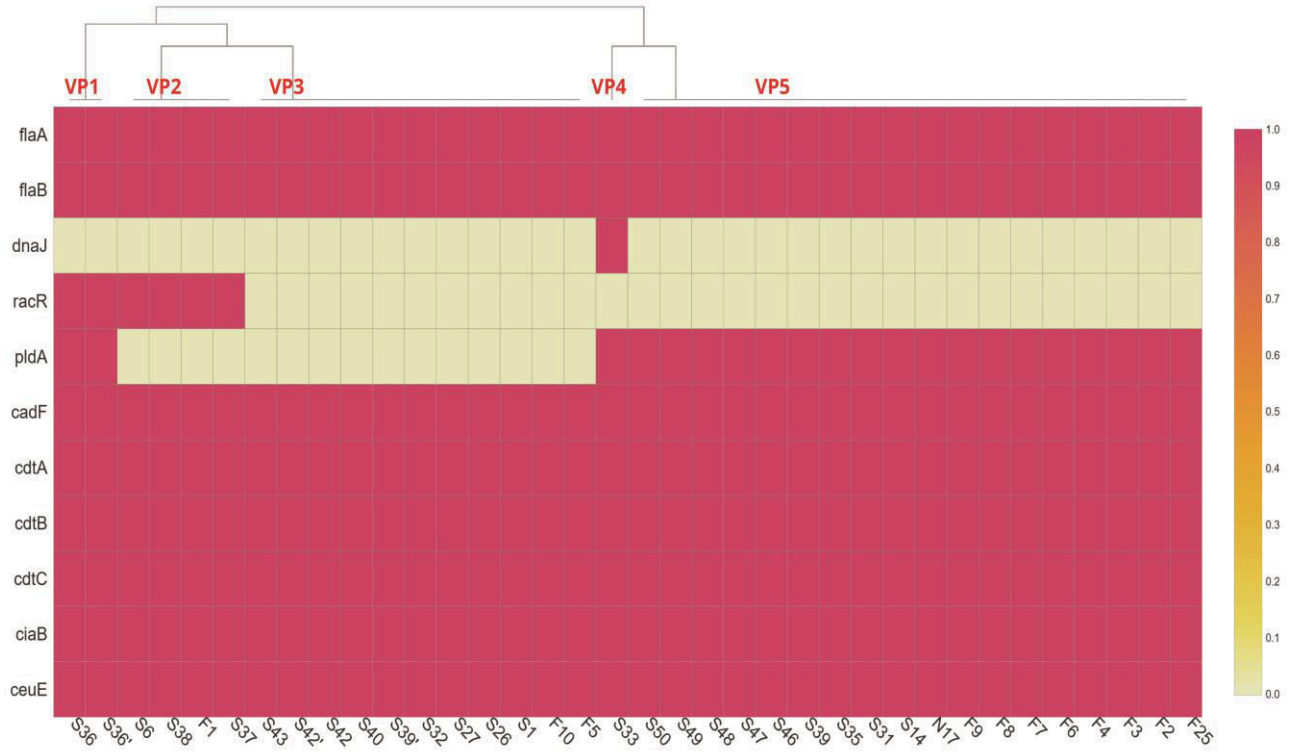

Fig. 4: Heat map showing the virulence genes profiles of *Campylobacter coli* isolated from chickens. Virulence profiles are marked in red bold font as VP1, VP2, VP3, VP4, and VP5

Table 4: Details of MLST of *C. coli* isolates and clonal complex

S. No.	Isolate ID	Type of chicken	Genes-Allele number							ST	CC
			aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*		
1	F1	Broiler	33	39	30	82	113	35	17	899	828
2	F25	Desi	124	39	30	79	113	43	17	10872	828
3	N17	Giriraja	33	39	30	82	113	44	17	872	828
4	S1	Broiler	33	39	30	82	908	47	17	9108	828
5	S6	Broiler	124	39	30	82	113	47	17	11031	828

ST: Sequence type, and CC: Clonal complex

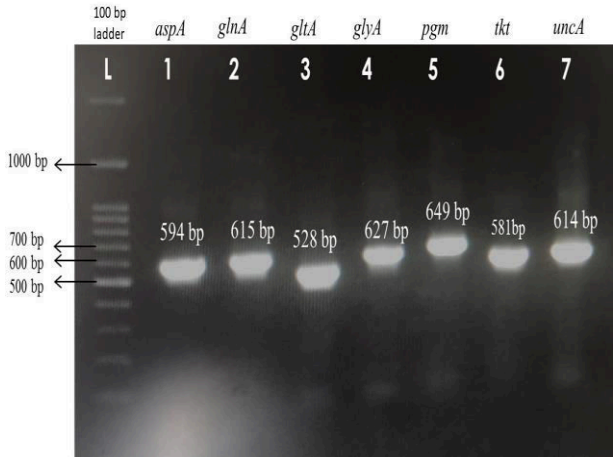

Fig. 5: Agarose gel showing PCR products of MLST genes - *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, and *uncA* of *C. coli* isolates

Table 5: Antimicrobial resistance profiles of *C. coli* isolates

S. No.	Antimicrobial resistance profile	No. of antimicrobials	No. of isolates
1	NAL	1	1
2	AZI-NAL-TET	3	1
3	AZI-GEN-NAL-TET	4	1
4	AZI-CIP-NAL-TET	4	1
5	CLI-ERY-NAL-TET	4	2
6	CLI-ERY-GEN-NAL-TET	5	2
7	AZI-CLI-ERY-NAL-TET	5	3
8	AZI-CIP-CLI-ERY-NAL-TET	6	2
9	AZI-CLI-ERY-GEN-NAL-TET	6	1

NAL: Nalidixic aid, AZI: Azithromycin, GEN: Gentamicin, CLI: Clindamycin, ERY: Erythromycin, TET: Tetracycline, and CIP: Ciprofloxacin

food poisoning. Conventional identification approaches are troublesome to some extent, since it necessitates fastidious growth criteria. Rapid diagnosis of *Campylobacter* spp. can be aided by molecular approaches such as multiplex PCR, and LAMP assay. Also, with the MLST system, the prevalent clone of *Campylobacter* in a region can be identified, and the sources of transmission could be traced. Additionally, the MIC is used to assess the sensitivity of *Campylobacter* spp. to various antimicrobials which will help the clinician to determine the drug dose for effective treatment of campylobacteriosis.

In the present study, the prevalence of *C. coli* (7%) was found in the districts of Tamil Nadu, India. Wieczorek *et al*. (2020) assessed the prevalence of *Campylobacter* spp. in chicken carcasses from 2014 to 2018 in Poland. Out of 2637 swab samples collected, 738 (28%) *C. coli* and 525 (22.2%) *C. jejuni* were isolated. Though *C. coli* isolates were more than *C. jejuni*, they were not predominant as observed in the present study. Walker *et al*. (2019) investigated the prevalence of *C. jejuni* and *C. coli* in retail chicken, beef, lamb, and pork products in Australia over two years. They found that *C. coli* were the most commonly isolated species from chicken meat, and offal. Whereas *C. jejuni* was primarily isolated from beef, lamb, and pork offal and meat. Few other studies also reported the predominance of *C. coli* over *C. jejuni* (Torralbo *et al*., 2015; Vinueza-Burgos *et al*., 2017; Rossler *et al*., 2020).

On the contrary, a study on the prevalence of *Campylobacter* spp. in chicken and chicken by-products in Japan revealed that out of 341 isolates, 278 (81.5%), and 63 (18.5%) were *C. jejuni*, and *C. coli*, respectively (Sallam *et al*., 2007). A very recent study by Wangroongsarb *et al*. (2021) also reported the predominance of *C. jejuni* (33.5%) over *C. coli* (18.2%). Prevalence of 38.6% and 24% of *C. jejuni* in chicken meat, and chicken intestine, respectively was recorded in Northern India (Khan *et al*., 2018).

However, the prevalence study of *Campylobacter* spp. in broiler chickens from rearing to slaughter in China revealed an almost equal prevalence of *C. jejuni* (66.3%), and *C coli* (60.4%) from 1534 samples (Tang *et al*., 2020).

In the present study, direct plating of caecal content yielded more *Campylobacter* isolates than plating after transportation in enrichment media. A similar experience was reported by Musgrove *et al*. (2001), who collected 128 caecal samples from broiler processing units, then plated them directly on Campy Cefex plates, and the same samples were also enriched in Bolton broth for 20 h at 42°C then plated on Campy Cefex agar plates. They could isolate *Campylobacter* spp. from all the directly plated agar plates (100%) but only from 63% of enriched caecal samples. These suggest that transportation in enrichment may lead to loss of *Campylobacter*, and direct plating is an efficient method for isolation of *Campylobacter* spp. However, Ugarte-Ruiz *et al*. (2012) reported that pre-enrichment step increased the isolation rate, and Kuana *et al*. (2008) reported that there was no statistical difference between the direct plating and enrichment method for isolation of *Campylobacter* spp.

There are several factors responsible for the survival and virulence of *Campylobacter* spp. Among the motility factors, Koolman *et al*. (2015) found that the prevalence of *flaA* was higher than that of *flaB*. However, in our study, both of these genes were present in all of the *C. coli* isolates. Gahamanyi *et al*. (2021) studied the virulence genes present in *C. jejuni*, and *C. coli* isolated from layer chickens in South Korea. They reported that the virulence genes *flaA*, *cdtB*, *cadF*, and *dnaJ* were found in all the *C. coli* isolates. In the present study, all the above-mentioned genes were present in all the *C. coli* isolates except *dnaJ* which was found in only one *C. coli* isolate.

Rossler *et al*. (2020) reported that *flaA* (100%), and *cadF* (92%) genes were highly prevalent in their *Campylobacter* spp. isolates which are similar to this study. Garcia-Sanchez *et al*. (2020) reported that *cdtA* and *cdtC* genes were absent in their *C. coli* isolates but were present in all *C. coli* isolates in the present study.

Out of five *C. coli* isolates MLST profiles, two new STs were found. However, all isolates belonged to ST-828 clonal complex. The exclusive predominance of ST-828 clonal complex was reported from Turkey by Kashoma *et al*. (2014), and Ecuador by Vinueza-Burgos *et al*. (2017). The presence of ST-899 in commercial broiler flocks was also reported by Ladely *et al*. (2017) in Georgia. ST-872 *C. coli* was reported from human

campylobacteriosis cases in Luxembourg (Mossong *et al*., 2016), but it was isolated from broiler chicken in this study. All these studies proved that the ST-828 clonal complex was found to be predominant among *C. coli* isolates all over the world, and this clonal complex ST-828 was found in *C. coli* isolated from different sources like poultry, ruminants, and even humans. Since there is no previous study on the MLST profile of Indian *Campylobacter* species neither from humans nor from animals, the significance of the ST profile of this study could not be ascertained, and we could not even validate our findings. Hence, to the best of our knowledge, this is the first MLST study of *Campylobacter* isolates in India.

Most of the *C. coli* isolates in the present study were highly resistant to nalidixic acid, tetracycline, erythromycin, and clindamycin. Nevertheless, the study of Khan *et al*. (2018) on the antimicrobial resistance of *C. jejuni* isolates from North India showed that 59.4% of isolates were resistant to tetracycline, and even lower resistance (6.9% to 8.9%) was observed against nalidixic acid, ciprofloxacin, erythromycin, gentamicin, and azithromycin. In this study, 100% resistance to nalidixic acid was found in all *C. coli* isolates. A study in Thailand also disclosed that *C. coli* isolates from retail chickens were 100% resistant to quinolones, and they showed 76.8% resistance to cyclines, 37.7% resistance to macrolides, 36.2% resistance to clindamycin, and 13% resistance to gentamicin (Wangroongsarb *et al*., 2021). Wieczorek *et al*. (2020) reported that *C. coli* isolates from chicken were 93.9% resistant to ciprofloxacin and 93.8% resistant to nalidixic acid. However, in the present study, *C. coli* isolates were 100% resistant to nalidixic acid and 78.5% sensitive to ciprofloxacin. The possible reason for this kind of resistance could be due to the point mutation in the Quinolone determining region (QRDR) of *gyrA* gene of *C. coli* and *C. jejuni*, which confers resistance only to nalidixic acid without conferring resistance to ciprofloxacin. Thr86Ala mutation was the one that bestowed resistance to only nalidixic acid, but not to ciprofloxacin (Bachoual *et al*., 2001; Jesse *et al*., 2006). The higher resistance of *Campylobacter* isolates to nalidixic acid and tetracycline might be due to their usage in feed.

We conclude that *C. coli* is more prevalent in broilers than *C. jejuni* in Tamil Nadu. The present study also demonstrated that *C. jejuni* and *C. coli* in chicken caecal samples from the slaughterhouse are indicative of the possibility of public health hazards. Hence, necessary precautions, and hygienic measures should be followed in slaughterhouses and chicken shops to prevent campylobacteriosis outbreaks. Though two new sequence types were identified in this study, all the five *C. coli* isolates belonged to the same clonal complex ST-828. To our knowledge, this is the first report with the characterization of *C. coli* isolates by MLST in India.

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Conflict of interest

The authors declare that they have no conflict of interest.

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