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Molecular Typing of Virulence and Antimicrobial Resistance Genes with Mutation Tracking of *gyr*A Gene of Fluoroquinolone-Resistant Strains of *Campylobacter* Isolated from Broiler Chickens

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Abstract

Campylobacter is the most common bacterial cause of gastroenteritis globally. A total of 182 fully identified strains of *Campylobacter* species (42 *C. coli* and 140 *C. jejuni*) collected from 6 broiler farms were subjected to studying the antimicrobial resistance pattern and molecular typing of virulence (*cad*F, *ctd*A, *dna*J *waa*C,*iam*, and *fla*) and antimicrobial resistance genes (bla_{OXA-61}, *gyr*A, *tet*A, *tet*O, and *IR*) with sequencing of *gyr*A region of one strain of fluroquinolones resistant *C. coli* and *C. jejuni*. The identified isolates were highly resistant to erythromycin and sulfamethoxazole-trimethoprim. Furthermore, both meropenem and imipenem were effective against the recovered isolates. The tested *C. jejuni* and *C. coli* strains had 100%, 83.3%, and 83.3% prevalence of *cad*F, *ctd*A, and *dna*J virulence genes, respectively, while *waa*C, *iam*, and *fla* genes couldn't be detected. The bla_{OXA-61} resistance gene was found in all of the *Campylobacter* spp. examined. Furthermore, *gyr*A, *tet*A, *tet*O, and *IR* resistance genes were found in 100%, 83.3%, 83.3%, and 66.7% of the retrieved *C. jejuni* strains, respectively. Likewise, resistance genes were found in 83.3%, 83.3%, 83.3%, and 66.7% of the retrieved *C. coli* strains, respectively. Approximately 58% (7/12) of the *Campylobacter* spp. recovered were MDR. Furthermore, 50% (3/6) of the *C. jejuni* strains recovered were MDR, while 66.7% (4/6) of the *C. coli* isolates recovered were MDR with MARI(0.22-0.55). For detection of mutations of the *gyr*A gene, the sequence data of two isolates (*C. jejuni* and *C. coli*) were analyzed against the reference sequence on the gene bank where the *C. jejuni* strain had six mutations, while the *C. coli* strain had twenty-three. The current findings suggest that MDR *Campylobacter* strains in poultry may be able to transmit highly virulent *Campylobacter* as a foodborne pathogen.

KEYWORDS
Broilers, Resistance genes, Antimicrobials, *C. coli*, *C. jejuni*

INTRODUCTION

Human acute bacterial diarrhea is most commonly caused by *Campylobacter* species (CDC, 2014). The most prevalent species responsible for human infections are *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), and poultry products, especially raw or undercooked chicken as major vector for these bacteria (Skarp *et al*., 2016).

Campylobacter species are common in the digestive tracts of wild and domesticated birds and mammals. Chicken meat products contaminated with this zoonotic disease during slaughtering and carcass processing are the primary source of human infections (EFSA, 2010). Poor handling or undercooked chicken cause most human *Campylobacter* infections (Doorduyn *et al*., 2010). In chickens, *Campylobacter* infections cause little or no clinical illnesses (Luangtongkum *et al*., 2006) .

Antimicrobial resistance has become a worldwide crisis due to their extensive usage in animal husbandry for growth promotion and disease control over many years (WHO, 2014). However, studies have shown a close relationship between animal antibiotics and resistance in humans (Marshall and Levy, 2011) by resistance transfer to humans from animal products (Chantziaras *et al*., 2014), so the EU has regulated poultry antibiotic use since 2006 (ESVAC, 2018). *Campylobacter* has developed fluoroquinolone (ciprofloxacin) and macrolide (erythromycin) resistance (Lehtopolku *et al*., 2011). Quinolone resistance is usually caused by amino acid changes in DNA-GyrAse and Topoisomerase IV A subunits (*Gyr*A and *Par*C) (Ruiz, 2003). Altered cytoplasmic quinolone uptake and transferable mechanisms of quinolone resistance (TMQR) also contribute to quinolone resistance (Lluque *et al*., 2017). In addition to this, it has been shown that CmeABC is responsible for the expulsion of macrolides from within the bacterial cytoplasm (Cagliero *et al*., 2006). Due to its growth-promoting characteristics, tetracycline has been reviewed for use in farm animal husbandry (Chopra *et al*., 1992). *Campylobacter* isolates from poultry have increased antibiotic resistance (EFSA, 2012). The transmissible plasmid *tet* (O) gene causes such resistance (Taylor and Courvalin, 1988). The absence of genotyping of ambient *Campylobacter* strains in Egypt may contribute to the rapid dissemination of antibiotic-resistant bacteria and genes among poultry and people (Ghoneim *et al*., 2020).

The genetic association between virulence and antibiotic resistance has been largely neglected, even though they frequently co-emerge (Bunduruș *et al*., 2023) .This study aimed to investigate the antibiogram of *Campylobacter* spp. isolated from chicken carcasses, virulence, and antimicrobial resistance genes with

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the sequencing of the *gyr*A resistance gene of quinolone for detection of mutations.

MATERIALS AND METHODS

Ethics Statement

The current study followed the recommendations made by ARRIVE. Animal Ethics Review Committee of Suez Canal University (AERC-SCU 2023053), Egypt, provided permission to all layers handling and experiments.

Data about broiler farms, collection of samples, and isolation of bacterial strains published previously in research with DOI: 10.21608/SCVMJ.2023.194348.1120

Bacterial strains

A total of 182 fully identified strains of *Campylobacter* species (42 *C. coli* and 140 *C. jejuni*) collected from 6 broiler farms were subjected to studying the antimicrobial resistance pattern and molecular typing of different genes with the sequencing of *gyr*A region of one strain of fluoroquinolones resistant *C. coli* and *C. jejuni*.

Antimicrobial susceptibility testing

Disk diffusion on Mueller–Hinton medium (Oxoid, Hampshire, UK) following CLSI guidelines used for antimicrobial susceptibility testing. The following antimicrobial agents were used (n=9); amoxicillin(AMX) (25 µg), amoxicillin-clavulanic acid (AMC) (30 µg), meropenem (MEM) (10 µg), imipenem (IPM) (10 µg), ciprofloxacin (CIP) (5 µg), sulfamethoxazole/trimethoprim(SXT) (25 µg), doxycycline (DOX) (30 µg), tetracycline (TE) (30 µg), and erythromycin(E) (15 µg). (Oxoid, UK). Multiple antibiotic resistance (MAR) indexes were evaluated using Krupperman's 1983 approach.

Detection of genes encoding virulence factors and AR (antimicrobial resistance)

DNA extraction of *Campylobacter* isolates was performed according to the manufacturer's instructions of the QIAamp DNA Mini Kit (QIAGEN, Germany). The thermal profiles and oligonucleotides primers for PCR (Table 1). PCR was performed to detect 6 virulence genes specific to *C. coli* and *C. jejuni*: *fla*A (motility); *cad*F, and *dna*J (cell adhesion); *cdt*A and *waa*C (production of cytotoxins); *iam* (invasion-associated) and 5 antimicrobial resistance genes: *tet*(O) and *tet*(A)(tetracycline resistance), *iR* region (the intergenic region between *cme*R and *cme*A of the *cme*A gene), bla_{OXA-61} (beta-lactam resistance) and *gyr*A gene (fluroquinolone resistance) (Table 1). PCR assays used positive control strains from the AHRI, Dokki, Giza, Egypt, while negative controls were reactions without DNA templates. Agarose gel electrophoresis (1.5%) stained with ethidium bromide 0.5-µg mL-1 separated and photographed the amplified PCR products.

*Gene sequencing and phylogeny of gyr*A *gene*

The *gyr*A gene was sequenced for two recovered *Campylobacter* isolates (*C. jejuni* and *C. coli*) with high resistance to fluoroquinolones (ciprofloxacin). The quinolone resistance-determining region (QRDR) of the *gyr*A subunit of the DNA *gyr*Ase enzyme was extracted with a QIAquick PCR-Product extraction reagent (QIAGEN Sciences Inc., Germantown, MD, USA). PCR products were purified with the aid of the QIAquick® Gel Extraction Kit (QIAGEN, Germany). The sequencing reactions were performed

Table 1. List of used primers in this study.

Gene function	Target gene	Primer sequence (5'-3')	Length of amplified Product	Reference
Campylobacter spp. confirmatory gene	*23S rRNA*	TATACCGGTAAGGAGTGCTGGAG ATCAATTAACCTTCGAGCACCG	650bp	Wang *et al*. (2002)
C. jejuni confirmatory gene	*C. jejuni hipO*	GAA GAG GGT TTG GGT GGT G AGC TAG CTT CGC ATA ATA ACT TG	735bp	Al Amri *et al*. (2007)
C. coli confirmatory gene	*C. coli asp*	GGT ATG ATT TCT ACA AAG CGA G ATA AAA GAC TAT CGT CGC GTG	500bp	
Virulence genes	*flaA*	AATAAAAATGCTGATAAAACAGGTG TACCGAACCAATGTCTGCTCTGATT	855bp	Datta *et al*. (2003)
	cadF	TTG AAG GTA ATT TAG ATA TG CTA ATA CCT AAA GTT GAA AC	400bp	Al Amri *et al*. (2007)
	Iam	GCGCAAATATTATCACCC TTCACGACTACTACTATGCGG	518bp	Wieczorek *et al*. (2012)
	cdtA	GGAAATTGGATTTGGGGCTATACT ATCAACAAGGATAATGGACAAT	165 bp	Bang *et al*. (2003)
	waaC	TAATGAAAATAGCAATTGTTCGT GATACAAAAATCACTTTTATCGA	971bp	Nahar Nahar and Bin Rashid (2018)
	dnaJ	ATTGATTTTGCTGCGGGTAG ATCCGCAAAAGCTTCAAAAA	177 bp	Chansiripornchai and Sasipreeyajan (2009)
Tetracycline resistant	*tetA*	TTCTCTATATCGGGCGGATCGTGGC CCACCCGAAGCAAGCAGGACCATG	700 bp	Gibreel *et al*. (2004)
	TetO	GGCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAGC	559 bp	
Resistance gene	*IR region*	TTG CCA ATT GGA TAG AAA ATA ATC TCG TAT TCC TTT TGA GAG ATT GC	770 bp	Duarte *et al. (*2016)
B-Lactams	bla_{OXA-61}	AGAGTATAATACAAGCG TAGTGAGTTGTCAAGCC	372 bp	Sierra-Arguello *et al. (*2015)
Quinolone resistant	*gyr*A	GATGGTTTAAAGCCTGTTCAT CGCCATACCTACAGCTATACC	423 bp	Lindmark *et al.* (2004)

with the BigDyeTM Terminator 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, US) and analyzed with the Applied BiosystemsTM 3130 Genetic Analyzer.

The obtained sequences were truncated, a consensus was generated, and they were analyzed using Uniport Ugene version 47.0 (Okonechnikov *et al*., 2012). Using the National Centre for Biotechnology Information's (NCBI) online basic local alignment tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi), sequences were analyzed. The nucleotide sequence was translated into all 6 possible frames using the Expasy protein translation tool (https://web.expasy.org/translate/). BLASTp was used to determine the similarity of the translated proteins. Consensus sequences were then submitted to GenBank and assigned accession numbers (OP272538 for *C. jejuni* and OP324647 for *C. coli*).

The *gyr*A gene protein sequences were aligned to reference genomes and other selected sequences were retrieved from the GenBank and uniport databases in fatsa format for phylogenetic analysis. Multiple Sequence Alignments (MSA) were performed with the same software and cluster omega algorithm (Sievers *et al*., 2011).

The phylogenetic tree was constructed using IQ-TREE (Nguyen *et al*., 2015), and the best model finder and 1000 bootstrap repeats were used to discover the most correct model using maximum likelihood. The tree was rescaled for site substitutions using branch length. The phylogenetic tree was displayed, manipulated, and annotated using iTOL (Letunic and Bork, 2021).

Statistical analysis

Pearson's correlation and Chi-square test were evaluated by RStudio (version 4.1.2) .The examination of the AR phenotype's connection with virulence and antimicrobial resistance genes. The association was statistically significant if $p < 0.05$.

RESULTS

Antimicrobial resistance testing

The antimicrobial resistance test of the retrieved *C. jejuni* isolates exhibited that the recovered strains were resistant to sulfamethoxazole-trimethoprim (100%), and erythromycin (66.7%). Moreover, the recovered isolates were sensitive to both meropenem and imipenem (100%), while *C. coli* isolates showed that the tested strains were resistant to sulfamethoxazole-trimethoprim (83.3%), erythromycin (83.3%) and amoxicillin (83.3%). Moreover, the recovered isolates were sensitive to both meropenem and imipenem (83.3%) as shown in Table 2 and Figure 1.

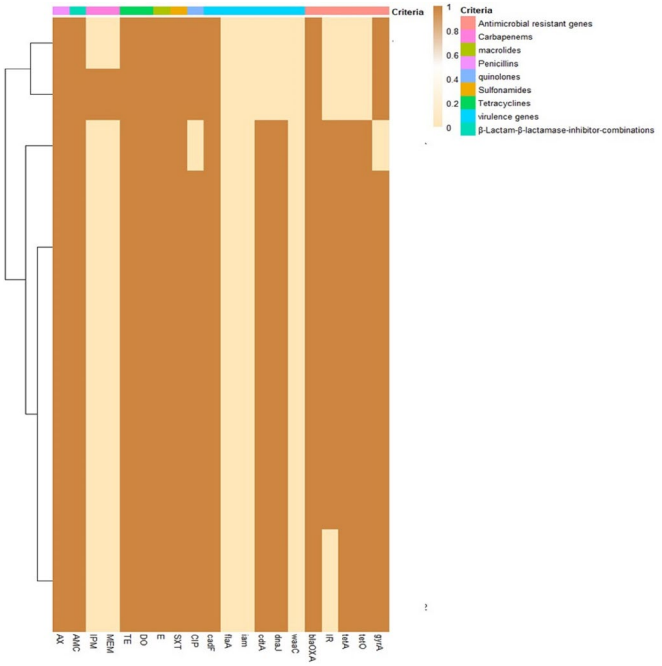

Fig. 1. A heatmap representing the isolates' antimicrobial resistance traits, virulence genes, and resistance genes. Dark brown squares show phenotypic resistance, intermediate sensitivity, and virulence and resistance genes; light brown squares indicate sensitivity and genes.

Virulence and resistance genes dissemination in the recovered Campylobacter serovars

In the present study, using PCR proved that the tested *C. jejuni* and *C. coli* strains harbored *cad*F, *ctd*A, and *dnaJ* virulence genes with a prevalence of 100%, 83.3%, and 83.3%, respectively. *waa*C,*iam*, and *fla* genes couldn't be amplified.

Concerning the distribution of the antimicrobial resistance genes, all the tested *Campylobacter* spp. (100%) harbored the bla_{OXA-61} resistance gene. Furthermore, the retrieved *C. jejuni* strains carried *gyr*A, *tet*A, *tet*O, and *IR* resistance genes with a prevalence of 100%, 83.3%, 83.3%, and 66.7%, respectively. Likewise, the retrieved *C. coli* strains carried *gyr*A, *tet*A, *tet*O, and *IR* resistance genes with a prevalence of 83.3%, 83.3%, 83.3%, and 66.7%, respectively (Table 3, Figure 1). Besides, the correlation co-

Table 2. The in-vitro antimicrobial susceptibility testing of the isolated *Campylobacter* spp.

Antimicrobial classes	Tested antimicrobial agents	Interpretation					
		C. jejuni			*C. coli*		
		Sensitive (%)	Intermediate (%)	Resistant (%)	Sensitive (%)	Intermediate (%)	Resistant (%)
Penicillins	Amoxicillin	50	0	50	0	16.7	83.3
β-Lactam-β-lactamase-inhibitor-combinations	Amoxicillin-clavulanic acid	0	100	0	0	83.3	16.7
Carbapenem	Meropenem	100	0	0	83.3	16.7	0
	Impenem	100	0	0	83.3	16.7	0
Macrolides	Erythromycin	0	33.3	66.7	0	16.7	83.3
Quinolones	Ciprofloxacin	0	66.7	33.3	16.7	66.7	16.7
Tetracycline	Tetracycline	0	83.3	16.7	0	66.7	33.3
	Doxycycline	0	50	50	0	66.7	33.3
Sulfonamides	Trimethoprim-Sulfamethoxazole	0	0	100	0	16.7	83.3

efficient was detected between the tested antimicrobial agents, virulence genes, and resistance genes as illustrated in Figure 2. The strong positive correlation between CIP and *gyr*A (r=1),*tet*O and *dnaJ*,*cad*F and (r=0.63), and *tet*A and *dnaJ*,*cad*F and (r=0.63).

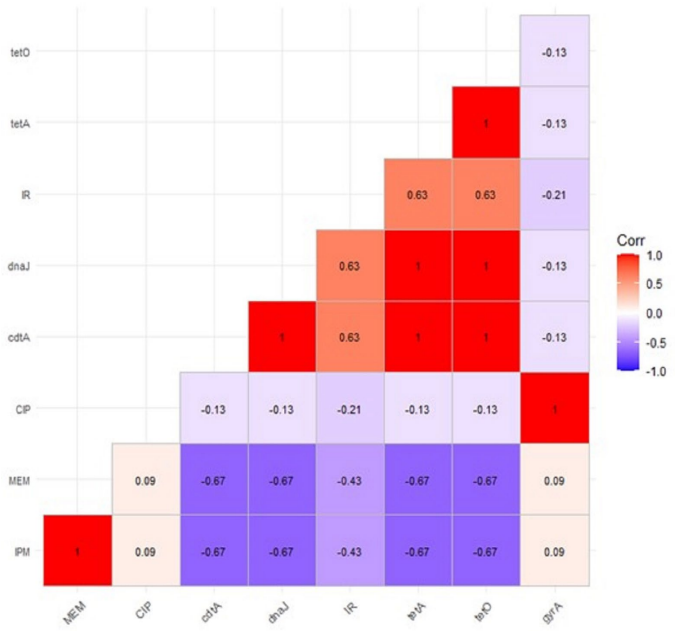

Fig. 2. Association between phenotypic resistant patterns, virulence genes and resistance genes among recovered isolates.

Multidrug resistance patterns of the retrieved Campylobacter strains

Our results evaluated that 58% of the retrieved *Campylobacter* spp. were MDR Moreover, 50% of the retrieved *C. jejuni* strains were MDR with MARI(0.22-0.55). Furthermore, 66.7% of the retrieved *C. coli* strains were MDR with MARI(0.22-0.55).

*Sequences of the gyr*A *Gene in Ciprofloxacin Resistant C. jejuni and C. coli*

Nucleotide analysis for the entire *gyr*A gene of *C. jejuni* was subjected for identity in comparison to 21 strains of *C. jejuni* deposited in the gene bank data where the identity ranged between 95.2% and 96%. Of these strains, RefSeq (Accession No: YP 002344422.1), 15 isolates are resistant to fluoroquinolone, 2 isolates are resistant to ciprofloxacin and 3 isolates are MDR. The difference in the nucleotide sequence is referred to the mutation in the *gyr*A gene of an isolate of the current study. This analysis involved 22 amino acid sequences. After removal of all the ambiguous positions for each sequence pair, the *C. jejuni* strain had 6 position mutations (Glu-59-Gln, Lys-68- Ile, Tyr-80-Phe, Thr-86-Ile, Asp-118-Ala, and Thr-126-Ile) in the QRDR fragment of the *gyr*A gene, as shown in Figures 3 and 4.

The *gyr*A sequence data of *C. coli* showed similarity with 7 QRDR and other Blastp resulted in hits of *C. coli* sequence data of *gyr*A gene isolated from chicken and other sources such as hu-

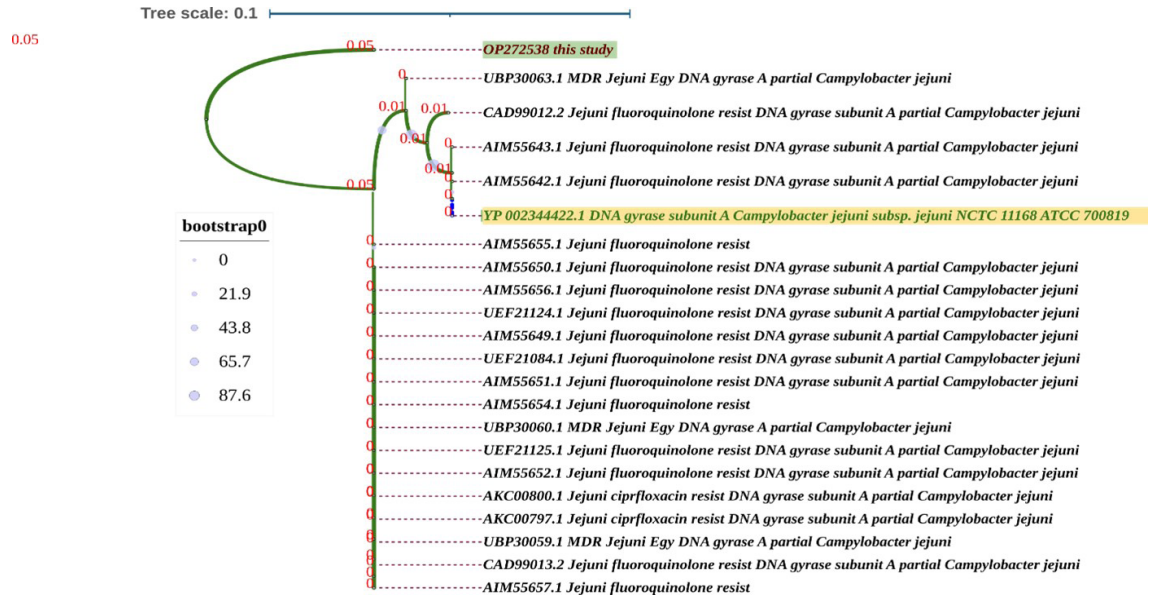

Fig. 3. Maximum likelihood phylogenetic tree The IQ-TREE with Model Finders phylogenetic tree for *C. jejuni*'s *gyr*A gene was based on fluoroquinolone-resistant alignment. Tree roots are in the middle. The accession number and short ID of each recovered sequence are assigned. Green indicates our isolates. From 1000 bootstrap repeats and branch length as visualized by iTOL, bootstrap values were determined.

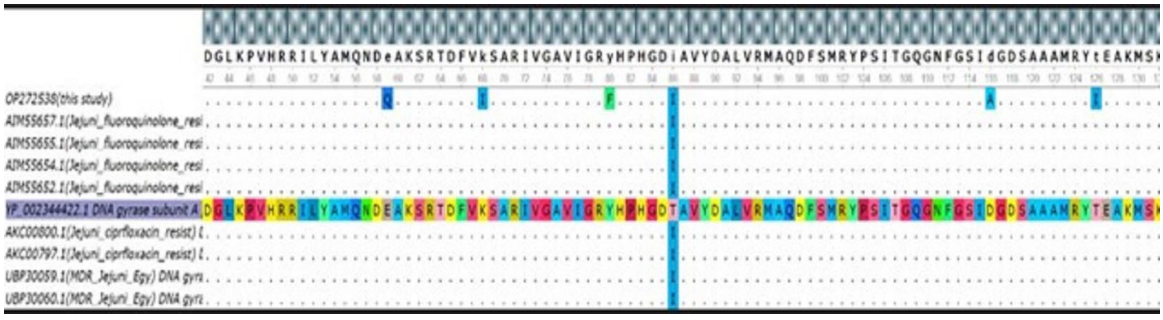

Fig. 4. *gyr*A Gene fragment MSA for the *C. jejuni* isolates compared to other isolates and strains as visualized against RefSeq by UGEN software. The dot (.) represents identity, while a single alphabet highlights a mutation position among sequences.

man, food, cow, swine from different countries in the world, the isolate sequence of this study have high similarity with this sequences with a percentage between 100% and 99% as in figure 5. The translation of the sequence revealed 23 mutations (Asn-56-Gln, Asp-57-Asn, Leu-58-Asp, Glu -59-Gly, Val-60-Ala, Gly-61-Lys, Ser-64-Thr, Ala-65-Asp, Tyr-66-Phe, Lys-67-Val, Asp-75-Ala, Lys-79-Arg, Asp-107-Thr, Gly-119-Ser, Arg-129-Lys, Thr-131-Ser, Ile-132-Lys, Ala-134-Ser, Glu-135-His, Arg-139-Lys, Asp-154-Gly, Met-156-Glu, Ala-164-Ser) as shown in Figures 5 & 6.

Table. 3. Distribution of virulence and resistance genes in the recovered *Campylobacter* spp.

Types	Genes	*C. jejuni* (%)	*C. coli* (%)
Virulence genes	*cadF*	100	100
	ctdA	83.3	83.3
	dnaJ	83.3	83.3
	waaC	0	0
	Iam	0	0
	fla	0	0
Antimicrobial resistance genes	bla_{OXA-61}	100	100
	*gyr*A	100	83.3
	tetA	83.3	83.3
	*tet*O	83.3	83.3
	IR	66.7	66.7

DISCUSSION

Thermophilic *Campylobacter*, including *C. jejuni* and *C. coli*, are commonly found in domestic fowl such as chickens, turkeys, ducks, and geese (Sahin *et al.*, 2015). Although thermophilic *Campylobacter*s are not considered a substantial threat to poultry, they possess considerable significance regarding food safety and public health. *C. jejuni* causes most human *Campylobacter*iosis, followed by *C. coli* (Gölz *et al.*, 2014). The main reservoir of thermophilic *Campylobacter* species is known to be the chicken intestines (Stern and Robach, 2003). Numerous studies have consistently identified poultry is the most prevalent method that *Campylobacter* is spread to humans through consuming contaminated meat. It has been reported that chicken sources account for 50% to 80% of human *Campylobacter*iosis cases (Sibanda *et al.*, 2018).

The emergence of antimicrobial-resistant strains is a significant factor that has undoubtedly played a role in the lack of success in controlling and ultimately eliminating *Campylobacter* contamination in poultry flocks. Indeed, similar to other infectious disorders caused by bacteria, the treatment for *Campylobacter* mostly relies on the use of antibacterial agents. The efficacy of these medications is compromised by the development of resistant strains, leading to treatment failures in animals and the dissemination of resistant strains in humans. The emergence of drug-resistant strains in the human population. The antimicrobial resistance test of the retrieved *C. jejuni* isolates exhibited that the recovered strains were resistant to sulfamethoxazole-trimethoprim (100%), and erythromycin (66.7%). Moreover, the recovered isolates were sensitive to both meropenem and imipenem

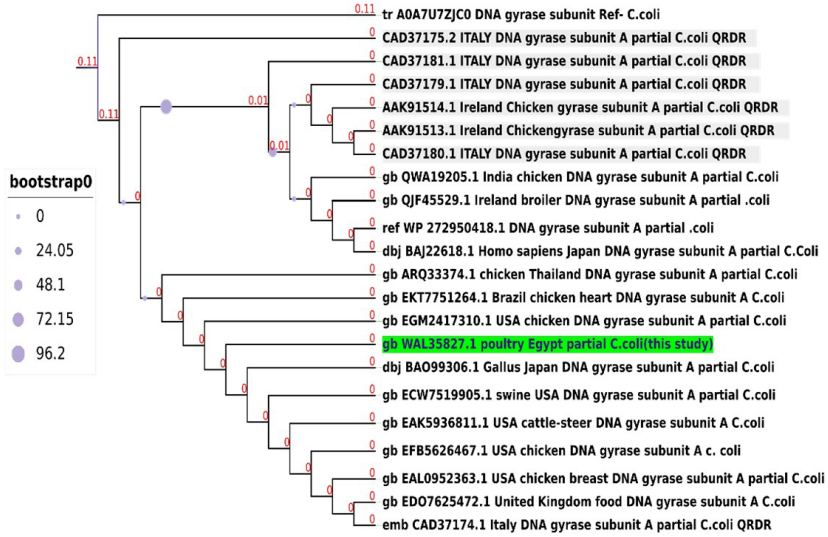

Fig. 5. maximum likelihood phylogenetic tree for *C. coli gyr*A gene, The tree was constructed in IQ-TREE with Model Finders. Tree roots are in the middle. The accession number and the brief ID of each recovered sequence are assigned. Our isolates are green, QRDR sequences are gray. From 1000 bootstrap repeats and branch length as visualized by iTOL, bootstrap values were determined.

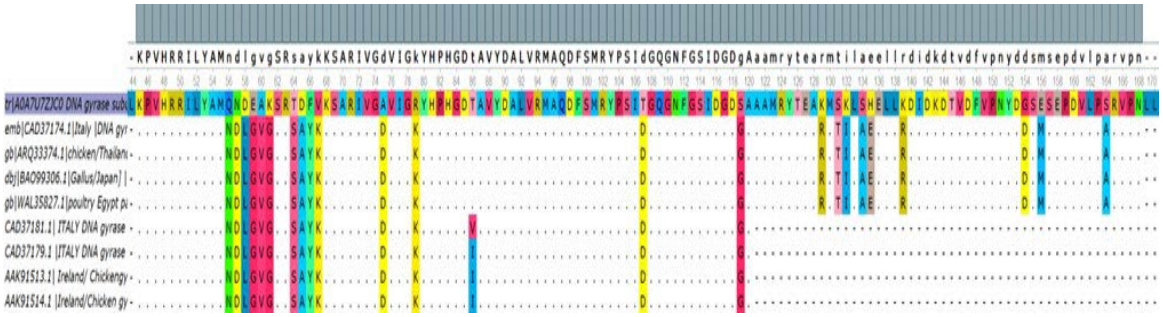

Fig. 6. *gyr*A Gene fragment MSA for the *C. coli* isolates compared to other isolates and strains as visualized against RefSeq. The dot (.) represents identity, while a single alphabet highlights a mutation site.

(100%), while *C. coli* isolates showed that the tested strains were resistant to sulfamethoxazole-trimethoprim, erythromycin, and amoxicillin with the same percent (83.3%). There was tetracycline resistance by 50 %, and 33.3 % for *C. jejuni* and *C. coli*, respectively. *C. jejuni* and *C. coli* isolates showed intermediate resistance to ciprofloxacin (quinolones) by 66.7% and resistance by 33.3% and 16.7% respectively. These results are consistence with (Gharbi *et al*., 2018) who reported high resistance of *Campylobacter* spp. isolated from broiler chicken in Tunisia against quinolones, macrolide, tetracycline, and chloramphenicol, ranging from 88.6% to 100%, and (Popa *et al*., 2022) recorded resistance of *Campylobacter* spp. Isolated from broiler chickens in Romania against ciprofloxacin (79.2%), tetracycline (49.5%), and streptomycin (7.9%) but the isolates were sensitive to gentamicin and erythromycin. Conversely, El-Adawy *et al*. (2015) reported that all isolates were susceptible to erythromycin. Luangtongkum *et al*. (2006); Luangtongkum *et al*. (2007); Luangtongkum *et al*. (2009) informed that *Campylobacter* is still sensitive to erythromycin. The differences between these results may indicate uncontrolled antibiotic use in veterinary medicine for therapeutic or preventive objectives. From 2016 to 2020, quinolone resistance developed, supporting this idea.

The current data detected high resistance of *C. jejuni* (100%) and *C. coli* (83.3%) against penicillins. Similarly, Gharbi *et al*., (2022) reported that the rates of β-lactams resistance of *Campylobacter* isolated from layers were 85.8% for ampicillin and 35.5% for amoxicillin/clavulanic acid. Messad *et al*. (2014) and Fraqueza *et al*. (2014) detected resistance rates concerning β-lactams by 100%. Extended-spectrum beta-lactamase (ESBL)-producing *Campylobacter* strains may spread ESBL-gram-negative bacteria. It is essential to remember that ESBL is an emerging threat to global health (Erb *et al*., 2018) .

The recovered isolates were sensitive to both meropenem and imipenem by 100% and 83.3% for *C. jejuni* and *C. coli* respectively. Carbapenems are considered to be a valuable class of antimicrobials for the treatment of difficult cases of *Campylobacter*iosis that do not exhibit a satisfactory response to initial therapeutic interventions (Nunes *et al*., 2023).

About 50% of *C. jejuni* and 66.7% of *C. coli* isolates were MDR. The results are in line with Fraqueza *et al*. (2014) who recorded that 74.4% of *Campylobacter* spp. isolated from broiler chickens in Portugal were MDR and higher than that of Popa *et al*. (2022) where 6.9% of isolates were MDR while Messad *et al*. (2014) and Gharbi *et al*. (2018) found that 100% of *Campylobacter* isolates from Algeria and Tunisia respectively were MDR. MDR *Campylobacter* spp. strains are increasingly isolated. *Campylobacter* spp. becomes AMR faster than other commonly isolated bacteria from animal-origin food and can be utilized for evaluating AMR in healthy domestic poultry (EFSA, 2021; Rivera-Gomis *et al*., 2021).

Thermophilic *Campylobacter* spp. are found everywhere, enabling them to spread between hosts and ecosystems. Determining *Campylobacter*iosis virulence markers is essential for identifying potentially more virulent strains and understanding infection pathways (Bunduruș *et al*., 2023). In the present study, using PCR proved that the tested *C. jejuni* and *C. coli* strains harbored *cad*F, *cdt*A, and *dnaJ* virulence genes with a prevalence of 100%, 83.3%, and 83.3%, respectively. Similar results were proved by (Gharbi *et al*., 2022) who detected *cdt*A gene in all *Campylobacter* isolates and stated that the *fla*A (100%–96%) was the most prevalent gene in *C. jejuni* and *C. coli*, respectively, followed by *cad*F (95%–89%), and *dnaJ* (50%–71%). However, the current data does not detect *waa*C, *iam*, and *fla*A genes.

Campylobacter's key virulence determinants are its capacity to attach via *cad*F, racR, virB11, pldA, and *dnaJ* genes, invade intestinal epithelial cells through ciaB and ceuE genes, produce toxin through *cdt*A, *cdt*B, *cdt*C genes, and survive in host cells (Bolton, 2015).

Antibiotic overuse in the chicken sector has raised serious concerns about the spread of resistant microbes (Hungaro *et al*., 2015; Luangtongkum *et al*., 2009). Ciprofloxacin, tetracycline, and erythromycins are considered the first-line antibiotics to combat bacterial infections in broiler farms which results in the upgrading of resistance of bacteria to these antibiotics. *Campylobacter* can acquire resistance through different mechanisms, including targ*et* alteration, drug inactivation, decreasing membrane permeability, and modification of the antibiotic efflux pumps (Yang *et al*., 2019).

The current results showed high resistance rates to several antibiotics among *Campylobacter* where all the tested *Campylobacter* spp. harbored the bla_{OXA-61} resistance gene (100%). Furthermore, the retrieved *C. jejuni* strains carried *gyr*A, *tet*A, *tet*O, and *IR* resistance genes with a prevalence of 100%, 83.3%, 83.3%, and 66.7%, respectively. Likewise, the retrieved *C. coli* strains carried *gyr*A, *tet*A, *tet*O, and *IR* resistance genes with a prevalence of 83.3%, 83.3%, 83.3%, and 66.7%, respectively. Phenotypically tetracycline-resistant *Campylobacter* isolates harbor *tet*(O) and *tet*(A) genes and this contributed to the long-term use of tetracyclines in poultry raising and food animal production which resulted in many tetracycline-resistant isolates in animal reservoirs (Béjaoui *et al*., 2022). The bla_{OXA-61} gene encoding resistance to β-lactams was detected in all tested isolates resistant to amoxicillin/clavulanic acid. There is a strong association between resistance to β-lactam drugs and the presence of the bla_{OXA-61} (Griggs *et al*., 2009; Matar, 2018).

Frasao *et al*. (2015) reported that resistance in *Campylobacter* spp. isolated from humans and animals started increasing after the authorization of fluoroquinolones in the veterinary field in 1990. The main mechanism of resistance to fluoroquinolones in *Campylobacter* species is mutations in the quinolone resistance determinant region (QRDR) of the *gyr*A gene, with the Thr-86-Ile mutation resulting in the replacement of the amino acid threonine with isoleucine becoming the main mutation in highly resistant strains.Thr-86-Ile mutation was recorded in the current study in *C. jejuni* which is in accordance with many studies in different countries (Frasao *et al*., 2015; Said *et al*., 2010; Wilson *et al*., 2000; Zirnstein *et al*., 2000). The alignment of *gyr*A gene of *C. jejuni* against the RefSeq (Accession No: YP 002344422.1) and fluoroquinolone-resistant strains showed 5 mutations in *C. jejuni* rather than Thr-86-Ile mutation and 23 mutations in *C. coli* . Qin *et al*. (2011); Iovine (2013) and Wieczorek and Osek (2013) reported other mutations rather than Thr-86-Ile such as Asp-90-Asn, Pro-104-Ser, Asp-85-Tyr but their role in fluoroquinolones resistance has not been established for these mutations. Frasao *et al*. (2015) reported 9 silent mutations in *Campylobacter* isolates from broiler and laying hens from Brazil rather than the Thr-86-Ile mutation. Moreover, the tested strains showed high genetic similarity with other *Campylobacter* strains isolated from diseased poultry in different regions, such as Spain, Egypt, Turkey and Slovenia and another source as human origin in Italy (CAD99012.2), cattle origin in turkey (UEF21084)for *C. jejuni* while *C. coli* strain showed high genetic similarity with other from poultry origin in different localities as Italy, USA, Brazil, Ireland, Egypt, India and Thailand and another sources human in Japan, swine and food in USA. Our findings are supported by Adiguzel *et al*. (2021), who reported that *Campylobacter* strains are genetically identical across sources. These studies also showed the epidemiological map and that poultry is a main vector for *gyr*A gene alterations, highlighting *Campylobacter*'s public health importance.

CONCLUSION

Virulent MDR *Campylobacter* species are widespread in broiler flocks, making eradication harder. Poultry is a reservoir and risk factor for MDR *Campylobacter* infection in humans, hence controlling the abuse of antimicrobial agents in poultry farms is crucial to preventing its spread in the environment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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