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Overview of Some Selected Virulence and Antibiotic Resistance Genes in *Campylobacter coli* Isolated from Broiler Chickens

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Abstract

Campylobacter coli is the more common zoonotic pathogen and poultry are the foremost blamed source of contamination. Therefore, this study tested 50 chicken pooled samples from apparently healthy and diseased broilers suffered from diarrhea and mortality from various broiler farms in the governorate of Kafr El-Sheikh which revealed 11 *Campylobacter coli* isolates with a percentage of 22%. The antimicrobial resistance profile showed high resistance to ampicillin and streptomycin with a percentage of 100% followed by 90.9% for kanamycin and oxytetracycline then cefotaxime with a percentage of 72.7% while, susceptibility was observed for amikacin, tobramycin and ciprofloxacin with the percentage of 100%, 72.7%, and 54.5%, respectively. Genotypically testing the virulence and antibiotic resistance genes; Virulence genes showed the highest percentage was 100% for *ciaB*, *flaA*, and *cdtC* followed by *VirB11* (n=10/11) 90.9% while, *dnaJ* and *pldA* were the lowest detection (9.1%) and (18.2%), respectively. Furthermore, the antibiotic resistance genes; *tetO* and *cmeB* were harbored in all isolates. In conclusion, *C. coli* isolates present in the investigated poultry has a multi-drug resistance appearance in combination with a high prevalence of virulence genes which may cause public health problems.

KEYWORDS
Campylobacter coli, Antibiotic sensitivity test, Virulence genes, Antibiotic resistance genes, Broiler chickens

INTRODUCTION

Globally, *Campylobacter* is a zoonotic pathogen, which is Gram-negative bacteria associated in foodborne diseases (Redondo *et al*., 2019). The two primary *Campylobacter* species that cause acute gastroenteritis in humans (Campylobacteriosis) are *Campylobacter jejuni* and *Campylobacter coli* (Chukwu *et al*., 2019). There were 246,571 cases of Campylobacteriosis in the EU in 2018 (EFSA and ECDC, 2019). According to reports, chicken and poultry byproducts are one of the main reservoirs and sources of human *Campylobacter* illnesses, which are carried by the creatures' gastrointestinal tracts (Wysok *et al*., 2015). The significant contamination of their carcasses in processing plants is due to *Campylobacter* colonization in the digestive tract of consumer chickens (Jeffrey *et al*., 2001). Nevertheless, *Campylobacter* infections in chickens rarely or never result in clinical illnesses (Luangtongkum *et al*., 2006). The cause of around 80% of human cases is poultry (EFSA, 2007; Mulder *et al*., 2020).

Pathogenesis of Campylobacteriosis contributed to different bacterial factors such as attachment, penetration, and the creation of poisons are what allow pathogens to infect the host, escape host defenses, and spread disease (de Oliveira *et al*., 2019). Moreover, virulence factors are at play, potentially more virulent strains exist which is identified molecularly (Redondo *et al*., 2019). *Campylobacter*'s ability to adhere is recognized through the genes *cadF*, *racR*, *VirB11*, *pldA*, and *dnaJ*, while its ability to invade intestinal epithelial cells is recognized through the genes *ciaB* and *ceuE*, and its ability to produce toxins is recognized through the genes *cdtA*, *cdtB*, and *cdtC* (Bolton, 2015). In addition, Wysok *et al*. (2020) found the genes for cytolethal distending toxin (*cdtA*, *cdtB*, and *cdtC*), cytolethal adhesion (*cadF*, *jlpA*, *porA*, and *pebA*), invasion (*ciaB*, *flaC*, and *pldA*), and T4SS (*VirB11*, virB10, virB9, *virB8*, *virB7*, *virB6*, *virB5*, *virB4*, and *virD4*). While, the *dnaJ* gene encodes a heat shock protein, which is associated with colonization (Eryildiz *et al*., 2020).

The primary factors contributing to an increase in the resistance rates among *Campylobacter* species are the abuse of antimicrobial medications in poultry husbandry to manage chicken diseases or to boost growth by adding antimicrobials to food (Raeisi *et al*., 2017) Drug resistance, however, is an international problem. Thus, the Centers for Disease Control (CDC) recommended testing for antibiotic resistance in *Campylobacter* isolates of human origin using drugs such as ciprofloxacin, erythromycin, tetracycline, and gentamicin (CDC, 2018). Antimicrobial agent resistance can be detected phenotypically or genetically (Su *et al*., 2019). The most common resistance strains to tetracycline are generally harbored *tet*(O)gene, which encodes a defense-related ribosomal protein (Laprade *et al*., 2016). Resistance to macrolides

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is activated by the CmeABC multidrug efflux pump (Iovine, 2013; Wieczorek and Osek, 2013). The disease transmission of *Campylobacter* in chicken businesses, as well as the molecular causes of antimicrobial resistance and virulence patterns, are poorly understood (Gharbi *et al*., 2022). Additional research revealed a strong association between virulence genes and antibiotic resistance in bacterial pathogens, pointing to a connection between resistance to antibiotics and these bacteria's propensity for colonization or invasion (Lapierre *et al*., 2016; Raeisi *et al*., 2017).

This study aimed to emphasize the presence of *Campylobacter coli* (*C. coli*) in chicken farms in the Kafr EL-Sheikh governorate and evaluate antibiotic resistance. It also undertaken to detect the presence of virulence and antibiotic-resistance genes, which primarily contribute to the pathogenicity of these bacteria.

MATERIALS AND METHODS

Sample collection

Fifty samples in total were gathered, under aseptic conditions from apparently healthy, diseased, and recently dead chickens from different Kafr EL-Sheikh governorate chicken farms. The organ samples were gathered as combined samples from the liver and intestine. Samples were immediately transported to the Kafr EL-Sheikh lab for *C. coli* isolation in an ice box.

Isolation and identification

Following ISO 10272-1: 2017 (ISO, 2017), *Campylobacter coli* was isolated and recognized as previously described. Briefly, samples were added to tubes with Bolton broth (1:10 w or v/v) as a pre-enrichment media at microaerophilic atmosphere condition which were incubated for 4-6 h at 37°C then incubated for 44.0±4.0 h at 42°C. Then plated on mCCDA media and incubated for 48 h at 41.5°C in a microaerophilic atmosphere. In the mCCDA agar, a typical *Campylobacter* colony appears greyish, had a metallic sheen, moist, flat, and had the propensity to spread. Suspected colonies were selected for Gram stain and microscopic examination of a wet mount to examine motility that showed motile curved bacilli with spiraling corkscrew motility also, appeared Gram-negative rods, curved, S- or spiral-shaped. Absence of aerobic growth at 25°C. in addition, biochemical tests proceed for all isolates and showed positivity to oxidase and catalase tests, and negative for hippurate hydrolysis.

Antimicrobial susceptibility pattern

Every isolate underwent an antibiotic sensitivity test (AST) using the disc diffusion method, as previously explained by WHO (2003) against 9 antibiotics (Oxoid, Basingstoke, UK), interpretation according to CLSI standard (2021).

Genotypic characterizations of Campylobacter coli

All isolates were at first subjected to polymerase chain response (PCR), particularly for the nearness of *ceuE* gene for confirmation of *C. coli*, identification of virulence genes: *dnaJ*, *ciaB*, *flaA*, *pldA*, virB 11, *cdtC*, and antibiotic resistance genes: *tetO* and *cmeB*, as described previously; briefly, DNA extraction was performed utilizing QIAamp DNA Smaller than expected pack (Qiagen, Hilden, Germany) concurring to the manufacturer's enlightening. Briefly, 200 µl of the test suspension was brooded with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After hatching, 200 µl of 100% ethanol was included in the lysate. The test was at that point was washed and centrifuged. The nucleic corrosive was eluted with 100 µl of elution buffer given within the pack. Preliminaries (Metabion, Germany) as appeared in Table 1, were utilized in a 25 µl response containing 12.5 µl of EmeraldAmp Max PCR Ace Blend (Takara, Japan), 1 µl of each preliminary of 20 pmol concentrations, 5.5 µl of water, and 5 µl of DNA layout. Enhancement was performed utilizing a Connected biosystem 2720 warm cycler and comprised of a beginning denaturation for 5 min. at 94°C taken after 35 cycles of denaturation: 94°C/30 s., strengthening: 42–58°C for diverse times, expansion: 72°C/ for diverse times; and a last expansion cycle at 72°C for 7-10 s. all subtle elements in Table 1.

The PCR items were observed by 1% agarose gel electrophoresis (AppliChem, Germany, GmbH) in 1x TBE buffer at room temperature utilizing angles of 5V/cm. For gel investigation, 20 µl of the uniplex PCR items were stacked in each gel space. A quality ruler 100 bp DNA stepping stool (Fermentas, Thermo, Germany) was utilized to decide the part estimate. The gel was captured

Table 1. Primers sequences, target genes, amplicon sizes and different cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Annealing	Extension	Final extension	Reference
ceuE	AAT TGA AAA TTG CTC CAA CTA TG TGA TTT TAT TAT TTG TAG CAG CG	462	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Shin and Lee (2009)
dnaJ	ATTGATTTTGCTGCGGGTAG ATCCGCAAAAGCTTCAAAAA	177	42˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Chansiripornchai and Sasipreeyajan (2009)
ciaB	TGC GAG ATT TTT CGA GAA TG TGC CCG CCT TAG AAC TTA CA	527	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Zheng *et al*. (2006)
flaA	TCCAAATCGGCGCAAGTTCA TCAGCCAAAGCTCCAAGTCC	217	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	
pldA	AAG AGT GAG GCG AAA TTC CA GCA AGA TGG CAG GAT TAT CA	385	46˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	
VirB11	TCTTGTGAGTTGCCTTACCCCTTTT CCTGCGTGTCCTGTGTTATTTACCC	494	53˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Datta *et al*. (2003)
cdtC	TGGATGATAGCAGGGGATTTTAAC TTGCACATAACCAAAAGGAAG	555	42˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Bang *et al*. (2003)
tetO	GGCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAGC	559	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Gibreel *et al*. (2004)
cmeB	CCTACCTCCTATACCTGG TTGAACTTGTGCCGCTGG	481	52˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	Pumbwe *et al. (*2004)

employing a gel documentation framework (Alpha Innotech, Biometra, USA) and the information was analyzed through a computer program.

RESULTS

Incidence of Campylobacter coli in broiler chicken samples

50 chicken pooled samples, including both seemingly healthy and sickly broilers who had diarrhea and mortality from several broiler farms in the Kafr El-Sheikh governorate, yielded a total of 11 *Campylobacter coli* isolates (22%).

Antibiotic susceptibility pattern of Campylobacter coli

Antimicrobial sensitivity test (AST) showed high resistance to ampicillin and streptomycin with a percentage of 100% followed by 90.9% for kanamycin and oxytetracycline then cefotaxime with a percentage of 72.7% while, susceptibility was observed for amikacin, tobramycin, and ciprofloxacin with a percentage of 100%, 72.7%, and 54.5%, respectively. As shown in Table 2 and Fig. 1.

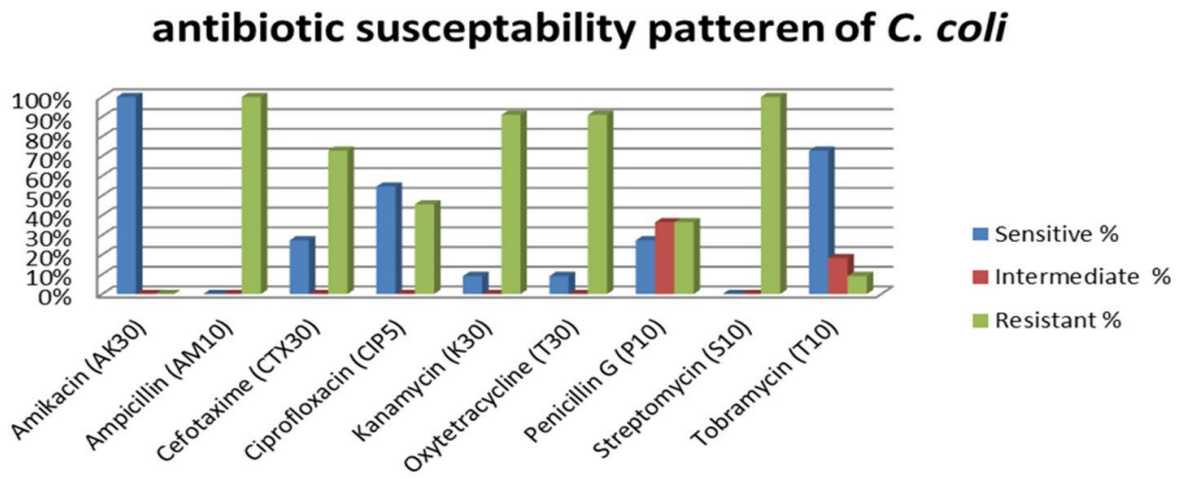

Fig 1. Antibiotic susceptibility pattern of *Campylobacter coli* isolates.

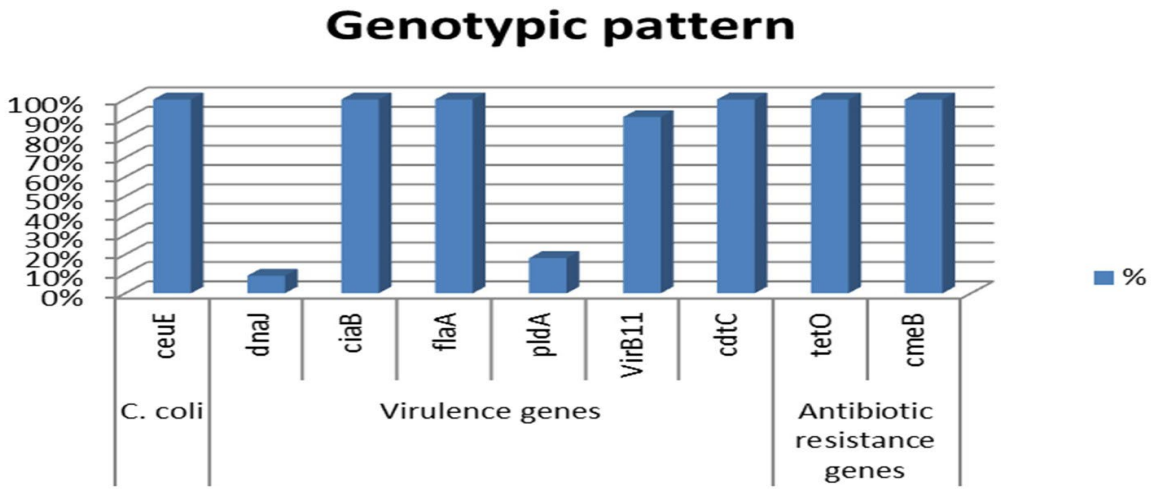

Fig 2. Genotypic pattern; Confirmation of *Campylobacter coli* isolates and Prevalence of some virulence and antibiotics resistance genes in *C. coli* isolates.

Table 2. Antimicrobial susceptibility of *Campylobacter coli* isolates.

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Amikacin (AK^{30})	1	100	0	0	0	0
Ampicillin (AM^{10})	0	0	0	0	11	100
Cefotaxime ($CTX3^{0}$)	3	27.3	0	0	8	72.7
Ciprofloxacin (CIP^{5})	6	54.5	0	0	5	45.5
Kanamycin (K^{30})	1	9.1	0	0	10	90.9
Oxytetracycline (T^{30})	1	9.1	0	0	10	90.9
Penicillin G (P^{10})	3	27.3	4	36.4	4	36.4
Streptomycin (S^{10})	0	0	0	0	11	100
Tobramycin (T^{10})	8	72.7	2	18.2	1	9.1

Genotypic characterizations of Campylobacter coli

All isolates were affirmed as *Campylobacter coli* by *ceuE* gene moreover, in the screening of virulence genes; the highest discovered genes were *ciaB*, *flaA*, and *cdtC* which were present in all isolates followed by virB 11(n=10/11). The lowest incidences were *dnaJ* and *pldA* were detected only once and twice, respectively, as shown in Table 3 and Figure 2. Furthermore, the antibiotic resistance genes; *tetO* and *cmeB* were harbored in all isolates.

Table 3. Confirmation of *Campylobacter coli* isolates and Prevalence of some virulence and antibiotics resistance genes in *C. coli* isolates.

Target	Genes	No. of positive result	%
C. coli	*ceuE*	11	100
Virulence genes	*dnaJ*	1	9.10
	ciaB	11	100%
	flaA	11	100
	pldA	2	18.20
	VirB11	10	90.90
	cdtC	11	100
Antibiotic resistance genes	*tetO*	11	100
	cmeB	11	100

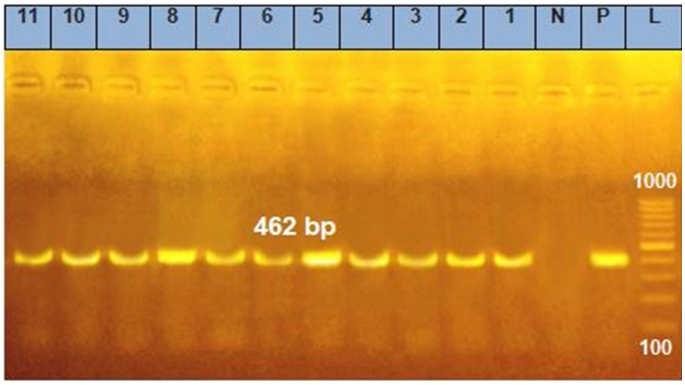

Fig 3. Agarose gel electrophoresis of PCR amplification products of *ceuE* gene for Confirmation of *C. coli*. Lane L: 100-1000 bp molecular size marker, Lane P: Control positive *C. coli ceuE* marker gene at 462 bp., Lanes 1, 2, 3, 4, 5, 6, 7,8,9,10 and 11: Positive *C. coli* strains for *ceuE* gene.

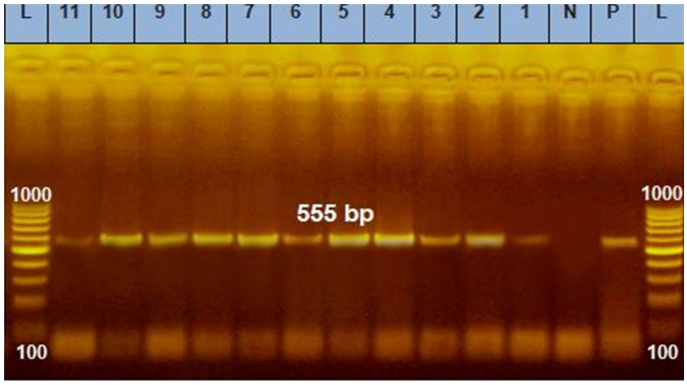

Fig 4. Agarose gel electrophoresis of PCR amplification products of *cdtC* virulence gene for characterization of *C. coli*.
Lane L: 100-1000 bp molecular size marker, Lane P: Control positive *C. coli cdtC* virulence gene at 555 bp., Lanes 1, 2, 3, 4, 5, 6, 7,8,9,10 and 11: Positive *C. coli* strains for *cdtC* gene.

DISCUSSION

Campylobacter could infect the flock or contaminate the chicken in processing which is usually transmitted to the full chain of production for poultry due to *Campylobacter* colonizing normally in broiler gut (Di Giannatale *et al*., 2019; Tang *et al*., 2020).

The detection rate of *Campylobacter coli* was 22% (11/50) from diagnosed broilers that were near Mohamed (2019) who showed 16.7% *C. coli* in chicken meat, in Egypt. Slightly higher findings were recorded in Poland, where *C. coli* was predominant with a percentage of 31.2% (Wieczorek *et al*., 2020) and 30.9% in Yangzhou, China (Huang *et al*., 2016). However, exceptions have been reported by Gahamanyi *et al.* (2021) who detected *C. coli* (10.9%) but *C. jejuni* was predominant from layer farms, in Korea. While, reported (8.3%) for layers in the United States and Sao Paulo, Brazil (Novoa Rama *et al*., 2018; Carvalho *et al*., 2013), respectively. In contrast, the Holland broiler samples exclusively contained *C. jejuni*. (Schets *et al*., 2017).

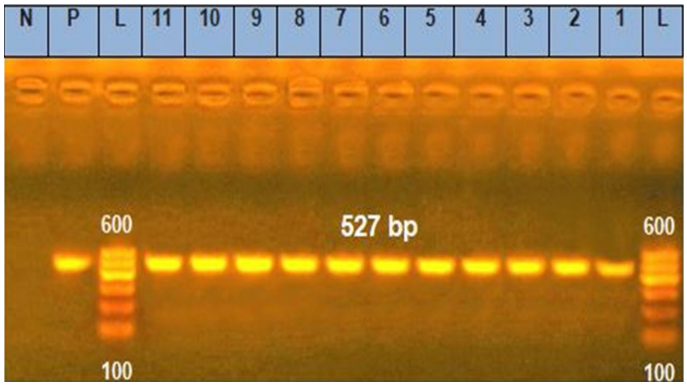

Fig 5. Agarose gel electrophoresis of PCR amplification products of *ciaB* virulence gene for characterization of *C. coli*.
Lane L: 100-600 bp molecular size marker, Lane P: Control positive *C. coli ciaB* virulence gene at 527 bp., Lanes 1, 2, 3, 4, 5, 6, 7,8,9,10 and 11: Positive *C. coli* strains for *ciaB* gene.

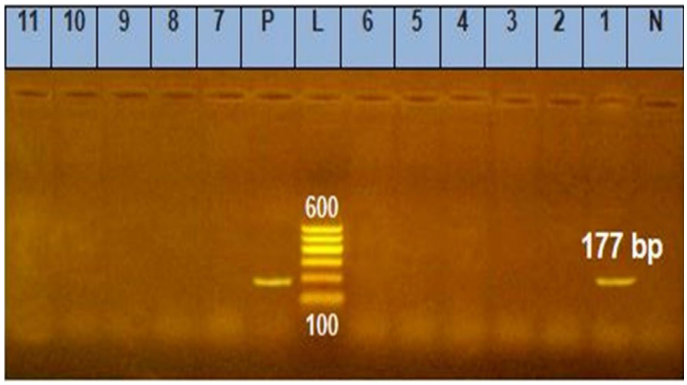

Fig 6. Agarose gel electrophoresis of PCR amplification products of *dnaJ* virulence gene for characterization of *C. coli*.
Lane L: 100-600 bp molecular size marker, Lane P: Control positive *C. coli dnaJ* virulence gene at 177 bp., Lanes 1: Positive *C. coli* strains for *dnaJ* gene.

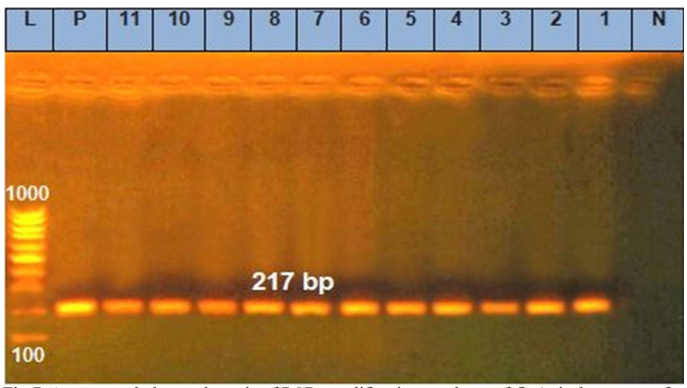

Fig 7. Agarose gel electrophoresis of PCR amplification products of *flaA* virulence gene for characterization of *C. coli*.
Lane L: 100-1000 bp molecular size marker, Lane P: Control positive *C. coli flaA* virulence gene at 217 bp., Lanes 1, 2, 3, 4, 5, 6, 7,8,9,10 and 11: Positive *C. coli* strains for *flaA* gene.

Antimicrobial resistance is a widespread issue that affects both human and animal health globally. Due to the widespread

use of antibiotics, the rise of antimicrobial resistance in the chicken industry is a growing problem (Luangtongkum *et al*., 2009; Hungaro *et al*., 2015; Redondo *et al*., 2019). The most frequently prescribed antimicrobial medications for the treatment of Campylobacteriosis are Macrolides, which include erythromycin and azithromycin. Tetracyclines, aminoglycosides, and fluoroquinolones are other therapeutic options (Wieczorek and Osek, 2013). Also, (Eryildiz *et al*., 2020) confirmed that macrolides and fluoroquinolones in addition to tetracycline, doxycycline, and chloramphenicol are commonly used in antimicrobial therapy to treat infections.

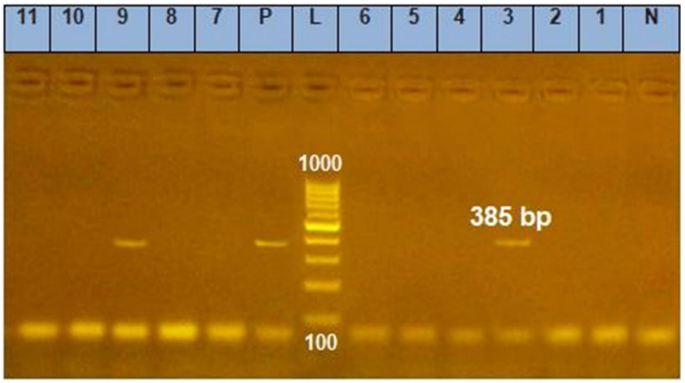

Fig 8. Agarose gel electrophoresis of PCR amplification products of *pldA* virulence gene for characterization of *C. coli*.
Lane L: 100-1000 bp molecular size marker, Lane P: Control positive *C. coli pldA* virulence gene at 385 bp., Lanes 3 and 9: Positive *C. coli* strains for *pldA* gene.

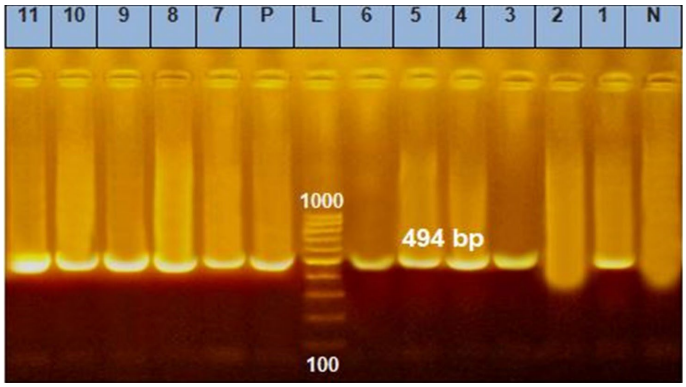

Fig 9. Agarose gel electrophoresis of PCR amplification products of *VirB11* virulence gene for characterization of *C. coli*.
Lane L: 100-1000 bp molecular size marker, Lane P: Control positive *C. coli VirB11* virulence gene at 494 bp., Lanes 1, 3, 4, 5, 6, 7,8,9,10 and 11: Positive *C. coli* strains for *VirB11* gene.

epidemiology due to the presence of virulome, which indicates adhesion, establishment, penetration, and production of toxins (Otigbu *et al*., 2018). *Campylobacter* spp. isolates from diarrheal samples were shown to have a higher prevalence of the cdt gene cluster than isolates from non-diarrheic samples (Wysok *et al*., 2020). The *flaA* gene participates in colonization, motility, autoagglutination, and biofilm formation, which contribute to *Campylobacter* disease (Guerry, 2007). Conversely, however, Gharbi *et al*. (2022) stated the *cmeB* gene was diagnosed for the incidence of multidrug resistance.

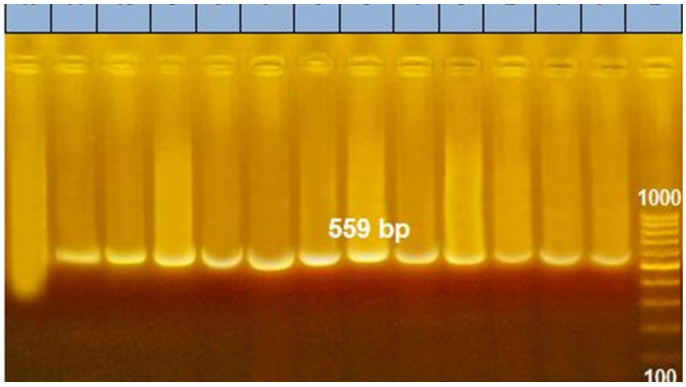

Fig 10. Agarose gel electrophoresis of PCR amplification products of *tetO* antibiotic resistance gene for characterization of *C. coli*.
Lane L: 100-1000 bp molecular size marker, Lane P: Control positive *C. coli tetO* antibiotic resistance gene at 559 bp., Lanes 1, 2, 3, 4, 5, 6, 7,8,9,10 and 11: Positive *C. coli* strains for *tetO* gene

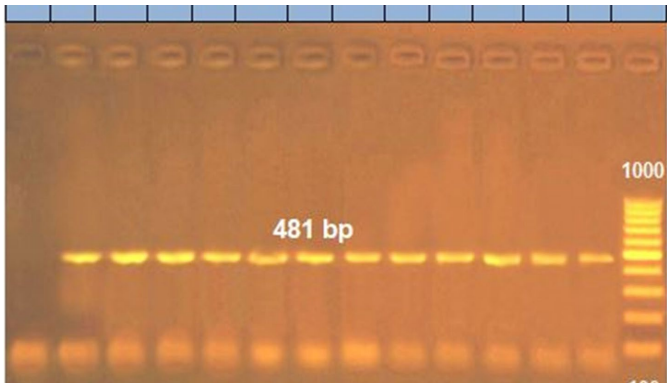

Fig 11. Agarose gel electrophoresis of PCR amplification products of *cmeB* antibiotic resistance gene for characterization of *C. coli*.
Lane L: 100-1000 bp molecular size marker, Lane P: Control positive *C. coli cmeB* antibiotic resistance gene at 481 bp., Lanes 1, 2, 3, 4, 5, 6, 7,8,9,10 and 11: Positive *C. coli* strains for *cmeB* gene

The antibacterial pattern of *C. coli* revealed 100% resistance to ampicillin and streptomycin, 90.9% resistance to kanamycin and oxytetracycline, 72.7% resistance to cefotaxime, and 100%, 72.7%, and 54.5% susceptibility to amikacin, tobramycin, and ciprofloxacin, respectively. Streptomycin, erythromycin, ampicillin, ciprofloxacin, and tetracycline were among the majority of them designated as critically important drugs for food animals by the World Organization for Animal Health (OIE) to safeguard the welfare and health of animals, help ensure the safety of food, and protect human health. (WOAH, 2021). According to another analysis, Korea's *Campylobacter* species had high levels of ciprofloxacin and tetracycline resistance. (Lee *et al*., 2017; Oh *et al*., 2017). In Egypt, for *C. coli* isolates, Ghoneim *et al*. (2020) found high resistance to nalidixic acid (71.4%), followed by tetracycline and erythromycin (57.1%), and finally ciprofloxacin and ceftriaxone (42.9%). Others reported that tetracycline, erythromycin, and (fluoro)quinolones have all been linked to human antimicrobial resistance (Zhu *et al*., 2006).

Their virulence is influenced by the virulome of the *Campylobacter* species (Han *et al*., 2019). In comparison to other intestinal bacteria, *Campylobacter* species have an elevated incidence and

This study confirmed that all isolates were *Campylobacter coli* by *ceuE* gene also, screening of virulence genes showed that all isolates were positive for *ciaB*, *flaA*, and *cdtC* followed by virB 11(n=10/11) 90.9%. The lowest incidences were *dnaJ* and *pldA* which were detected only once (9.1%) and twice (18.2%), respectively. Furthermore, the antibiotic resistance genes; *tetO* and *cmeB* harbored in all isolates. Close to the obtained results, Gharbi *et al*. (2022) stated that *flaA*, *cadF*, *racR*, and *VirB11* were frequently present over 87% up to 97%, followed by 100% for *ciaB* and cdt. Additional studies note that most of the isolates were found to include associated virulence genes that are linked to the pathogen adhesion, colonization, and invasion features. These genes included *flaA*, *cadF*, *racR*, *VirB11*, *ciaB*, and *pldA* (Redondo *et al*., 2019). The *flaA*, *flaB*, *ciaB*, *cdtA*, and *cdtC* genes were present in all the *C. coli* isolates. But only 80% of *C. coli* isolates had the *cdtB* and *pldA* genes, and only 20% had the *dnaJ* gene in human samples in Turkey (Eryildiz *et al*., 2020). Moreover, the molecular typing methods, *Campylobacter* of poultry origin was responsible for 80% of cases of human Campylobacteriosis (Newell *et al*., 2011). But all the investigated isolates had *flaA*, *cadF*, and *dnaJ*, according to Gahamanyi *et al*. (2021). Using the

gene *tetO*, Ghoneim *et al*. (2020) examined the molecular causes of tetracycline resistance in *Campylobacter* isolates. Overall, the research showed that tetracycline-resistant isolates did not all carry the *tetO* gene. Moreover, Obeng *et al*. (2012) found little association between tetracycline resistance and *tetO* gene expression. On the other hand, Wieczorek and Osek (2013) linked the *tetO* gene to significant levels of phenotypic resistance to tetracycline. The obtained results demonstrated a strong link between the *tetO* gene's existence and the phenotypic level of antibiotic resistance. In addition, Gharbi *et al*. (2022) indicated *tetO* and *cmeB* genes with percentages of 80 and 100 %, respectively in Tunisia. According to certain studies (Ghunaim *et al*., 2015), resistant strains were more severely invaded by susceptible strains than vice versa, while other studies point to the potential for susceptible strains to produce more severe infections than resistant strains (Feodoroff *et al*., 2009).

CONCLUSION

Campylobacter coli isolates in the present study has a multidrug resistance appearance in combination with a high prevalence of virulence genes which may cause public health problems.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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