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Prevalence of Virulence Genes and Antimicrobial Resistance Patterns of *Campylobacter* Species Isolated from Chicken in Egypt

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ABSTRACT

The high incidence of *Campylobacter* diarrhea represents a significant public health hazard. A total of 400 internal organs of either apparently healthy or diseased chicken were investigated for campylobacteriosis. The incidence of *Campylobacter* was higher in case of fecal samples, followed by fecal samples then duodenum samples. The highest recovery rates were obtained during summer season followed by the winter and spring, respectively. *Campylobacter* isolates were confirmed to be *C. jejuni* or *C. coli* using MapA and Col 2, 3 primers. The prevalence of virulence (*cadF*, *flaA*, *ceuE*) and CDT toxin (*cdtA*, *cdtB*, *cdtC*) encoding genes in *C. jejuni* and *C. coli* isolated from chicken illustrated that, 100, 78.6, 50 and 35.7% of *C. jejuni* isolates showed amplified fragments of *ceuEj*, *cadF*, *cdtabc* and *flaA* genes, respectively. Among the *C. coli* isolates 100% showed amplified fragments of *ceuEc*, no isolates had *cdtabc* genes, while 83.3% of *C. coli* isolates showed amplified fragments for *cadF* and *flaA* genes, respectively. Antibiotic resistance and plasmid virulence factors of *Campylobacter* were also estimated and all *C. jejuni* and *C. coli* isolates were totally susceptible to amikacin and tobramycin. Meanwhile high rate of antimicrobial resistance recorded may pose serious danger to the public health.

Key words: Campylobacter jejuni, *Campylobacter coli*, PCR, virulence genes, plasmid, antibacterial sensitivity

INTRODUCTION

Campylobacter jejuni is responsible for bacterial food borne gastroenteritis in many countries, usually due to the consumption of undercooked poultry (Keller and Shriver, 2014). In addition to gastrointestinal forms, 1% of cases may develop peripheral neuropathies including Guillain-Barré syndrome, reactive arthritis and functional bowel diseases, such as irritable bowel syndrome (Di Giannatale *et al*., 2014). Birds are the preferred host for *C. jejuni*, so the consumption or handling of poultry meat is a major risk factor of human campylobacteriosis (Harris *et al*., 1986). Virulence genes were known to be important for the virulence of *Campylobacter* species. They are important for both adherence and invasion of intestinal epithelial cells and a leading cause of bacterial gastroenteritis (Tracz *et al*., 2005). The *cadF* gene encodes a 37 kDa outer-membrane protein, functions as an adhesin responsible for certain steps of invasion, also, *cdtA*, *cdtB* and *cdtC* genes of *Campylobacter* species are necessary for the cytotoxic activity known to be lethal for host enterocytes (Rozynek *et al*., 2005). No evidence was seen for the involvement of plasmids in the virulence of *C. jejuni* until Bacon *et al*. (2000) who identified plasmid pVir in strain 81-176, pVir

is a ≈37.5 kb plasmid that contains components of a type IV secretion system (T4SS) known to be important for the virulence of a number of major bacterial pathogens. They suggested that the pVir plasmid is important *in vitro* for both adherence and invasion of intestinal epithelial cells in culture. Antibiotic treatment of campylobacteriosis will depend on the causative species, as observed in case of erythromycin, which is commonly used to treat *C. jejuni* infections. *C. coli* strains are most likely to be resistant to this antibiotic (Cloak and Fratamico, 2002). Therefore, the aim of the present study was to detect and characterize *C. jejuni* and *C. coli* strains isolated from chickens in Egypt and to establish its antibacterial and evaluate the virulence factors as well as comparing the genetic differences between the isolates by estimating the prevalence of virulence (cadF, flaA, ceuE) and CDT toxin encoding genes (cdtA, cdtB, cdtC) in *C. jejuni* and *C. coli* isolated from chicken.

MATERIALS AND METHODS

Specimens: A total of 400 samples (200 cecum, 80 duodenum, 40 bile and 80 fecal samples) were collected from 200 apparently healthy and 200 diarrheic chickens from various markets for isolation of *Campylobacter* species. The samples were cultured within 2 h.

Isolation and Identification of *Campylobacter* species: According to Boonmar *et al.* (2005), 1 g of each sample was added to 9 mL of preston *Campylobacter* selective enrichment broth (Oxoid) supplement with *Campylobacter* selective supplement SR 117 (Oxoid), *Campylobacter* growth supplement SR 84 (Oxoid) and 5% defibrenated horse blood. The inoculated broth was incubated under microaerophilic conditions at 42°C for 24 h. Then According to El-Jakee *et al.* (2008) the enrichment culture was streaked onto *Campylobacter* blood-free selective agar base (Oxoid) supplemented with *Campylobacter* selective supplement SR 155 (Oxoid) and incubated under microaerophilic conditions at 42°C for 3-5 days. Suspected *Campylobacter* colony confirmed using standard biochemical procedure including catalase, oxidase and hippurate hydrolysis tests (Nachamkin, 2003).

PCR assay: Campylobacter isolates were analyzed using Polymerase Chain Reaction technique (PCR) according of Yang *et al.* (2003) using easy Quick™ DNA extraction kit (Genomix). PCR primers were commercially synthesized as shown in Table 1. Amplification was performed in a total reaction volume of 50 µL containing 5.0 ng DNA/µL, 40 pmol of each primer, 1 U of *Taq* DNA polymerase, 200 µM each dATP, dCTP, dTTP and dGTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5.5 mM MgCl2 and nuclease free water up to 50 µL. After overlaying the mixture with mineral oil,

Table 1: Oligonucleotide primers used for characterization of *Campylobacter* isolates

Genes	Primer sequence	Annealing temp. (°C)	Amplicon (bp)	References
mapA	5'CTATTTTTTTGAGTGCTTGTG3' 5'GCTTTATTTGCCATTTGTTTTATTA3'	55	589	El-Jakee *et al.* (2008)
$Col_{2.3}$	5'AATTGAAAATTGCTCCA CTATG3' 5'TGATTTTATTATTAGCAGCG3'	53	462	Manfreda *et al.* (2003)
ceuEj	5'CATGCTACGGTAAAAGTTCTGC 3' 5'GATCTTTTTGTTTTGTGCTGC3'	40	793	Talukder *et al.* (2008)
ceuEc	5' AAG AAA AAA TAT TCA TTT GCA3' 5'ATT TTA TTT GAA GCA GCG 3'	44	894	Gonzalez *et al.* (1997)
flaA	5´ATGCGATTTCTTAAAAC3´ 5´CTGTAGTAAACATTTATTTTG3´	50	1713	Muller *et al.* (2006)
cadF	5´TTGAGGGTAAATAGATG3´ 5´CTAATACCTAAAGTTGAAAC3´	41	400	Muller *et al.* (2006)
cdtABC	5´GGAAATTGGATTTGGGGCTATACT3´ 5´TTGCACATAACCAAAAGGAAG3´	50	1215	Rozynek *et al.* (2005)

the tubes were placed in the thermocycler and the amplification was performed under the following program: Initial denaturation at 94°C for 5 min followed by 34 cycles of denaturation at 94°C for 30 sec, annealing steps were variable according to primers (*mapA* and $Col_{2,3}$ genes according to Manfreda *et al.* (2003); *ceuEj* gene according to Talukder *et al*. (2008); *ceuEc* gene according to Gonzalez *et al.* (1997); *flaa* and *cadf* gene according to Muller *et al.* (2006) and *cdtABC* gene according to Rozynek *et al.* (2005) for 30 sec and extension at 72°C for 1 min. A final extension step was done at 72°C for 10 min. PCR products were electrophoresed at 100 V for 60 min in a 1.5% agarose gel stained with ethidium bromide (0.2-0.25 mg mL^{-1}) (Sambrook *et al*., 1989). Standard marker GeneRulerTM 10 kb DNA Ladder (Fermentus) was used. Gels were visualized under uv-lighter.

Plasmid profile analysis: The plasmids of the isolates were extracted using Biospin Plasmid DNA Extraction Kit, China.

Antibiotic resistance test: Antibiotic sensitivity test was conducted according to the method described by the National Committee for Clinical Laboratory Standard (NCCLS., 2000). Mueller Hinton agar medium (Oxoid) with 5% defibrinated horse blood was used. The antimicrobial susceptibility test disks (Oxoid) used in this study were: Amikacin (30 μg), Amoxicillin (20 μg), Ampicillin (10 μg), Cephalothin (30 μg), Cepholax (30 μg), Ciprofloxacin (5 μg), Deoxycycline (30 μg), Erythromycin (15 μg), Gentamycin (10 μg), Nalidixic acid (30 μg), Norfloxacin (10 μg), Spiramycin (10 μg), Streptomycin (10 μg), Tetracycline (30 μg), Tobramycin (10 μg), Trimethoprim (1.25 μg) plus Sulfamethoxazole (23.75 μg).

RESULTS

Prevalence of *Campylobacter* species among the examined samples: It is clear from Table 2 that out of the examined 200 apparently healthy chickens, 2 isolates were identified as *C. jejuni* and 2 isolates were identified as *C. coli* with an incidence of 1% each. Meanwhile *C. jejuni* (12 isolates) and *C. coli* (4 isolates) were isolated from the examined 200 diarrheic chickens with an incidence of 6 and 2%, respectively. The highest recovery rates were obtained during summer season followed by the winter and spring, while no campylobacters could be isolated in autumn.

Confirmation of *Campylobacter* isolates by polymerase chain reaction: Identification of *Campylobacter* isolates by polymerase chain reaction was performed using *mapA* and $col_{2,3}$

Table 2: Incidence of *Campylobacter* species among the examined samples

	C. jejuni		*C. coli*		Total	
Source of the isolates	No.	%	No.	%	No.	%
Apparently healthy chickens (n = 200)	2	1	2	1	4	2
Cecum contents (n = 100)	1	1	2	2	3	3
Duodenum samples (n = 40)	-	-	-	-	-	0
Bile samples (n = 20)	-	-	-	-	-	0
Fecal swabs (n = 40)	1	2.5	-	-	1	2.5
Diarrheic chickens (n = 200)	12	6	4	2	16	8
Cecum contents (n = 100)	8	8	1	1	9	9
Dudenum samples (n = 40)	1	2.5	1	2.5	2	5
Bile samples (n = 20)	-	-	-	-	-	0
Fecal swabs (n = 40)	3	7.5	2	5	5	25
Total (n = 400)	14	3.5	6	1.5	20	5

primers. All *C. jejuni* isolates produced amplified fragment at 589 bp using mapA primer, while no amplified fragment could be produced from *C. coli* isolates as shown in Fig. 1. Among $col_{2,3}$ primers, all *C. coli* isolates produced amplified fragment at the expected positions (462 bp), while no amplified fragment could be produced from *C. coli* isolates as shown in Fig. 2.

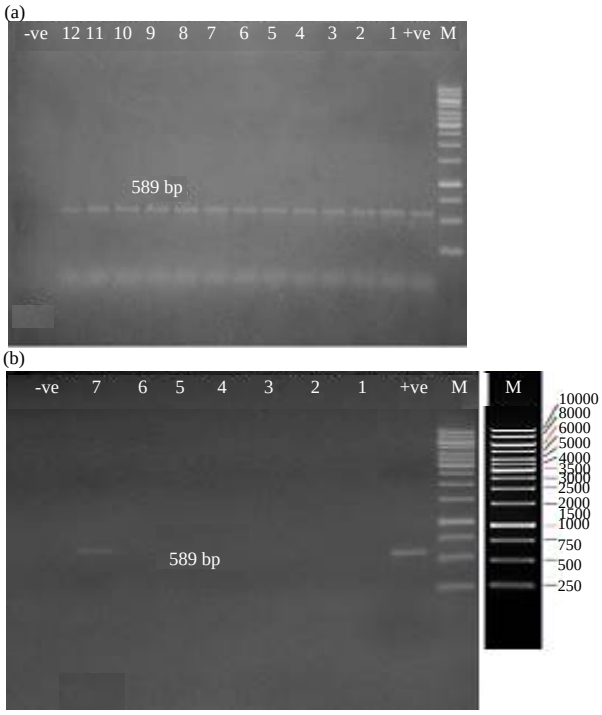

Fig. 1(a-b): CR assay showing the amplification of 589 bp using mapA primers among the *C. jejuni* isolated from chickens, (a) Lane M: GeneRuler^{TM} 10 kb DNA Ladder (Fermentus), Lane +ve: *C. jejuni* reference strain ATCC 33291, Lanes 1-12: *C. jejuni* isolates and Lane -ve: *C. coli* reference strain ATCC 33559 and (b) Lane M: GeneRuler^{TM} 10 kb DNA Ladder (Fermentus), Lane +ve: *C. jejuni* reference strain ATCC 33291, Lane -ve: *C. coli* reference strain ATCC 33559, Lanes 1-6: *C. coli* isolates and Lane 7: *C. jejuni* isolates

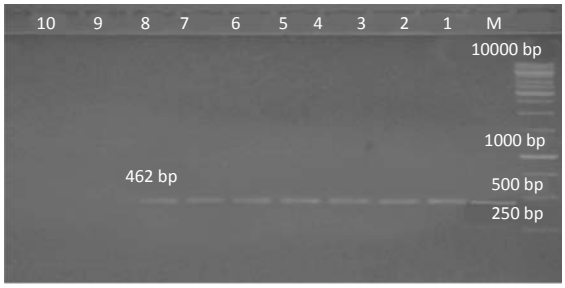

Fig. 2: CR assay showing the amplification of 462 bp using $col_{2,3}$ primers among the *C. coli* isolated from chickens. Lane M: GeneRuler^{TM} 10 kb DNA Ladder (Fermentus), Lane 1: *C. jejuni* reference strain ATCC 33291, Lane 2: *C. coli* reference strain ATCC 33559, Lanes 3-8: *C. coli* isolates, Lanes 9 and 10: *C. jejuni* isolates

Detection of some virulence and toxin genes among the isolates: Results of PCR detection of virulence and toxin genes among *C. jejuni* and *C. coli* isolated from chickens are shown in Table 3 and Fig. 3-7, respectively. It is clear that 100, 78.6, 50 and 35.7% of *C. jejuni* isolates

Table 3: *Campylobacter* isolates carrying genes and plasmids

	C. jejuni (n = 14)		*C. coli* (n = 6)		Total (n = 20)	
Virulence genes and plasmids	No.	%	No.	%	No.	%
ceuEj	14	100.0	-	0.0	14	70
ceuEc	-	0.0	6	100.0	6	30
flaA	5	35.7	5	83.3	10	50
cadF	11	78.6	5	83.3	16	80
cdtabc	7	50.0	-	0.0	7	35
Plasmid under 2500 bp	9	64.3	1	16.7	10	50
Plasmid over 2500 bp	10	71.4	-	0.0	10	50
Plasmid 0ver 5000 bp	-	0.0	1	16.7	1	5
Plasmid over 10000 bp	10	71.4	2	33.3	12	60

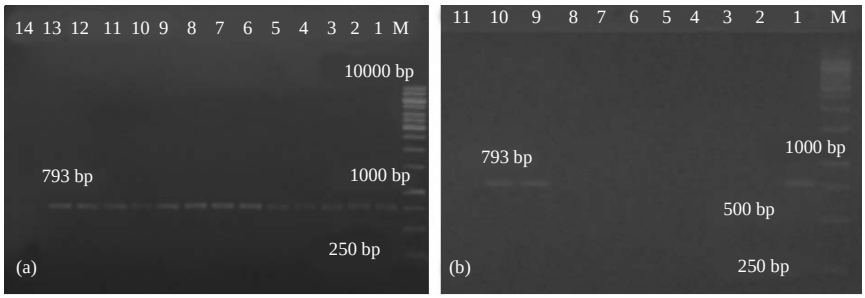

Fig. 3(a-b): Representative PCR assay showing the result of using *ceuEj* primers among the *Campylobacter* isolated from chickens, (a) Lane M: GeneRuler™ 10 kb DNA Ladder (Fermentus), Lane 1: *C. jejuni* reference strain ATCC 33291, Lane 2-13: *C. jejuni* isolates, Lane 14: *C. coli* reference strain ATCC 33559 and (b) Lane M: GeneRuler™ 10 kb DNA Ladder (Fermentus), Lane 1: *C. jejuni* reference strain ATCC 33291, Lanes 2-8: *C. coli* isolates, Lanes 9 and 10: *C. jejuni* isolates, Lane 11: *C. coli* reference strain ATCC 33559

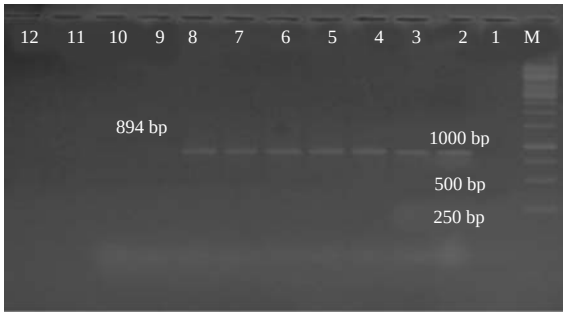

Fig. 4: Representative PCR assay showing the result of using *ceuEc* primers among the *Campylobacter* isolated from chickens. Lane M: GeneRuler™ 10 kb DNA Ladder (Fermentus), Lane 1: *C. jejuni* reference strain ATCC 33291, Lane 2: *C. coli* reference strain ATCC 33559, Lanes 3-8: *C. coli* isolates and lanes 9-12: *C. jejuni* isolates

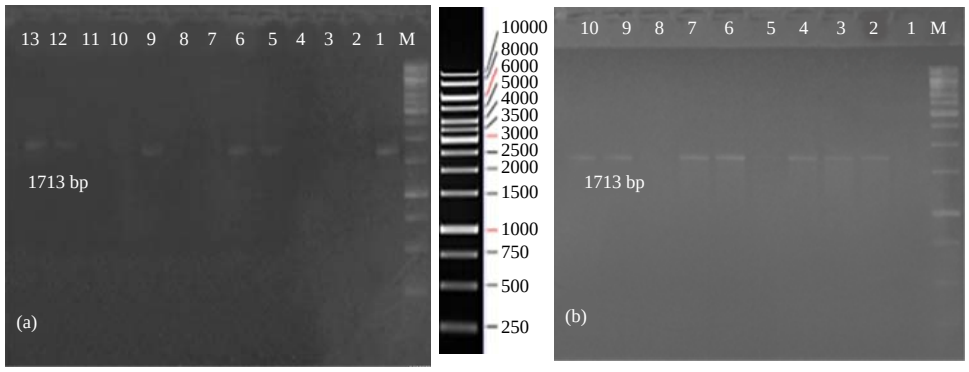

Fig. 5(a-b): Representative PCR assay showing the result of using *FlaA* primers among the *Campylobacter* isolated from chickens, (a) Lane M: GeneRuler™ 10 kb DNA Ladder (Fermentus), Lane 1: *C. jejuni* reference strain ATCC 33291, Lanes 2-13: *C. jejuni* isolates (Lanes 5, 6, 9, 12 and 13 had *FlaA* gene) and (b) Lane M: GeneRuler™ 10 kb DNA Ladder (Fermentus), Lane 1: *C. coli* isolate, Lane 2: *C. coli* reference strain ATCC 33559, lane 3: *C. jejuni* reference strain ATCC 33291 and Lanes 4-10: *C. coli* isolates (Lanes 4, 6, 7, 9 and 10 had *FlaA* gene)

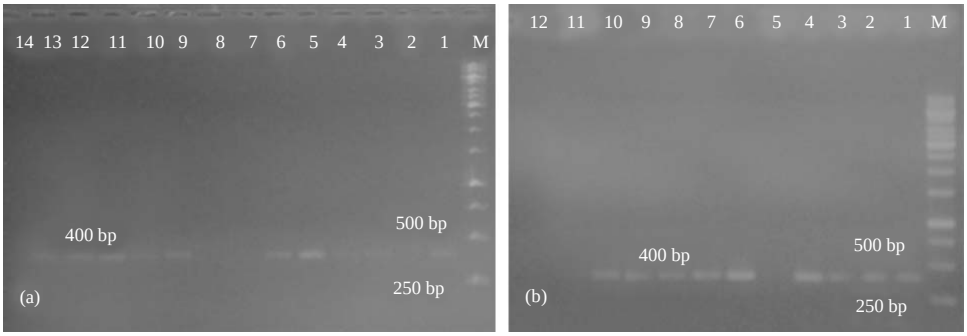

Fig. 6(a-b): Representative PCR assay showing the result of using *cadF* primers among the *Campylobacter* isolated from chickens, (a) Lane M GeneRuler™ 10 kb DNA Ladder (Fermentus) and lanes 1-14*: C. jejuni* isolates (Lanes 1, 3, 4, 5, 6, 9, 10, 11, 12 and 13 had *cadF* gene) and (b) Lane M: GeneRuler™ 10 kb DNA Ladder (Fermentus), Lanes 1-5: *C. jejuni* isolates and lanes 6-12: *C. coli* isolate. Lanes 1, 2, 3, 4, 6, 7, 8, 9 and 10 had *cadF* gene

showed amplified fragments at 793, 400, 1215 and 1713 bp specific for *ceuej*, *cadf*, *cdtabc* and *flaA* genes, respectively and no isolate had *ceuEc* gene. No *C. coli* isolates had neither *ceuEj* nor *cdtabc* genes, while 83.3% of the isolates showed amplified fragments at 400 and 1713 bp specific for cadF and *FlaA* genes (each), respectively. One hundred percent of *C. coli* isolates showed amplified fragments specific for *ceuEc* at 89.4 bp.

Detection of *Campylobacter* isolates carrying plasmid: As shown in Table 3 and Fig. 8, 71.4, 64.3 and 71.4% of *C. jejuni* carrying plasmids over 2.5 kbp, under 2.5 kbp and over 10 kbp,

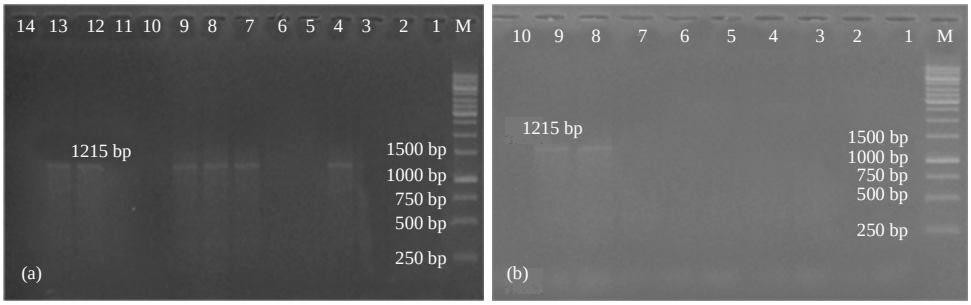

Fig.7(a-b): Representative PCR assay showing the result of using *cdtABC* primers among the *Campylobacter* isolated from chickens, (a) Lane M: GeneRuler^{TM} 10 kb DNA Ladder (Fermentus) and Lanes 1-14: *C. jejuni* isolates (Lanes 3, 4, 7, 8, 9, 12 and 13 had *cdtABC* gene) and (b) Lane M: GeneRuler^{TM} 10 kb DNA Ladder (Fermentus), Lanes 1-6: *C. coli* isolates and Lanes 7-10: *C. jejuni* isolates (Lanes 8 and 9 had cdtABC gene)

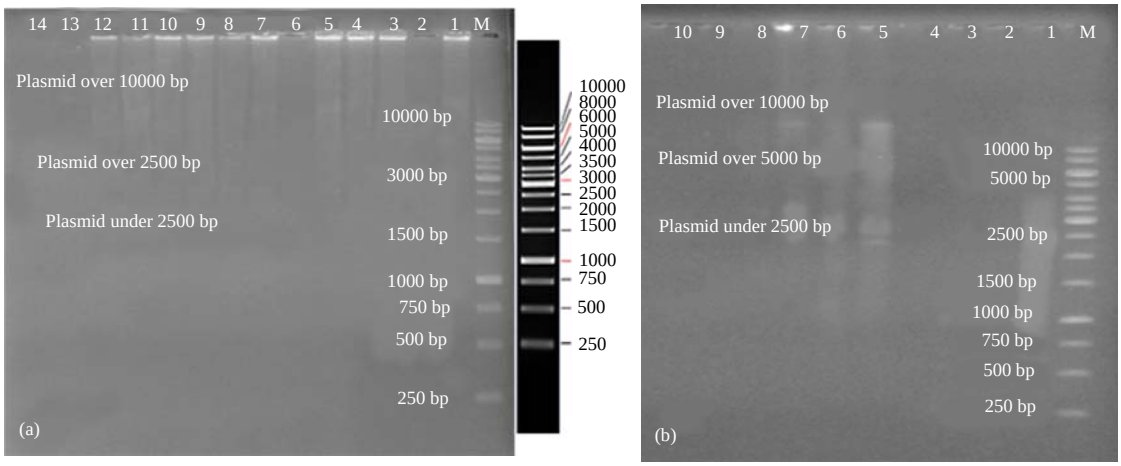

Fig. 8(a-b): Detection of plasmids among the *Campylobacter* isolated from chickens, (a) Lane M: GeneRuler^{TM} 10 kb DNA Ladder (Fermentus) and Lanes 1-12: *C. jejuni* isolates, Lane 13: *C. coli* reference strain ATCC 33559 and Lane 14: *C. jejuni* reference strain ATCC 33291 and (b) Lane M: GeneRuler^{TM} 10 kb DNA Ladder (Fermentus), Lanes 2-7: *C. coli* isolates, Lanes 1 and 8: *C. jejuni* isolates, Lane 9: *C. coli* reference strain ATCC 33559 and Lane 10: *C. jejuni* reference strain ATCC 33291

respectively. Among the *C. coli* isolates no isolates carrying plasmids over 2.5 kbp, 16.7% of the *C. coli* isolates had plasmids under 2.5 kbp and also over 5 kbp (each), while 33.3% of *C. coli* isolates had plasmids over 10 kbp.

Antimicrobial sensitivity test of *Campylobacter* isolates: Table 4 and 5 showed that, all *C. jejuni* and *C. coli* isolates were susceptible to amikacin and tobramycin (100% each). Also the isolates showed sensitivity to gentamicin, nalidixic acid, ciprofloxacin, norfloxacin, spiramycin and streptomycin with a percentage varied between (100-50%.). On the other hand a high level of

Table 4: Antimicrobial susceptibility patterns of *C. jejuni* isolates (n = 14)

	Sensitive		Intermediate		Resistant	
Antimicrobials	No	%	No	%	No	%
Amikacin	14	100.0	0	0.0	0	0.0
Amoxicillin	4	28.6	0	0.0	10	71.4
Ampicillin	4	28.6	0	0.0	10	71.4
Cephalothin	3	21.4	0	0.0	11	78.6
Cepholax	0	0.0	0	0.0	14	100.0
Ciprofloxacin	13	92.9	0	0.0	1	7.1
Doxycycline	3	21.4	0	0.0	11	78.6
Erythromycin	6	42.9	2	14.3	6	42.9
Gentamycin	13	92.9	0	0.0	1	7.1
Nalidixic acid	14	100.0	0	0.0	0	0.0
Norfloxacin	13	92.9	1	7.1	0	0.0
Spiramycin	7	50.0	1	7.1	6	42.9
Streptomycin	8	57.1	0	0.0	6	42.9
Tetracycline	2	14.3	1	7.1	11	78.6
Tobramycin	14	100.0	0	0.0	0	0.0
Trimethoprim sulfamethoxazole	2	14.3	0	0.0	12	85.7

Table 5: Antimicrobial susceptibility patterns of *C. coli* isolates (n = 6)

	Sensitive		Intermediate		Resistant	
Antimicrobials	No	%	No	%	No	%
Amikacin	6	100.0	0	0.0	0	0.0
Amoxicillin	2	33.3	0	0.0	4	66.7
Ampicillin	2	33.3	1	16.7	3	50.0
Cephalothin	0	0.0	0	0.0	6	100.0
Cepholax	0	0.0	0	0.0	6	100.0
Ciprofloxacin	3	50.0	1	16.7	2	33.3
Doxycycline	1	16.7	1	16.7	4	66.7
Erythromycin	4	66.7	0	0.0	2	33.3
Gentamycin	6	100.0	0	0.0	0	0.0
Nalidixic acid	3	50.0	2	33.3	1	16.7
Norfloxacin	3	50.0	1	16.7	2	33.3
Spiramycin	4	66.7	0	0.0	2	33.3
Streptomycin	4	66.7	1	16.7	1	16.7
Tetracycline	1	16.7	0	0.0	5	83.3
Tobramycin	6	100.0	0	0.0	0	0.0
Trimethoprim+sulfamethoxazole	1	16.7	0	0.0	5	83.3

resistance was recorded to cepholax (100% each), amoxicillin (71.4 and 66.7%), trimethoprim sulfamethoxazole (85.7 and 83.3%), ampicillin (71.4 and 50%) among *C. jejuni* and *C. coli* respectively. It is clear that *C. coli* isolates had 100% resistant to cephalothin. Also *C. coli* isolates were resistant to tetracycline and trimethoprim sulfamethoxazole (83.3% each). As well as *C. coli* isolates showed (66.7%) resistance to amoxicillin and doxycycline (each) while it showed (50%) resistance to only ampicillin.

DISCUSSION

According to the recent European Food Safety Authority report, the most frequently reported zoonotic disease in humans in the European Union in 2008 was *Campylobacter* infection (Wieczorek and Osek, 2011). Poultry are considered the main carrier of *Campylobacter* species and serve as major sources of infection to human. In fact, the consumption of inadequately cooked poultry meat and its incorrect handling are the main source of infection for humans (Lee and Newell, 2006).

In the present investigation a total of four hundred samples collected from apparently healthy (n = 200) and diarrheic (n = 200) chickens were examined bacteriologically for isolation of *Campylobacter* species. Twenty samples were found to be bacteriologically positive for campylobacters with an incidence of 5%. *Campylobacter jejuni* (n = 14) and *C. coli* (n = 6) were identified from the examined samples. An extensive epidemiological study was performed by Torralbo *et al*. (2014) to determine the prevalence of *Campylobacter* infection in cloacal swabs and environmental swabs from broiler flocks in Spain, the flock prevalence was 62.9%. They added that the flocks were predominantly infected by *C. jejuni* and *C. coli* but were also infected by untyped *Campylobacter* spp. and mixed-species infection could be found.

The overall prevalence of *Campylobacter* species was 9.2% for all wild birds sampled (n = 781) and *Campylobacter jejuni* was the most prevalent species (8.1%), while *Campylobacter coli* and *Campylobacter lari* prevalence estimates were 1.4 and 0.3%, respectively (Keller and Shriver, 2014). Variations in isolation rates may be due to several reasons, such as differences in local prevalence of *Campylobacter* in the specific region, management of chicken, environment, sampling techniques employed, seasonality and laboratory methodologies employed.

Several PCR-based assays have been developed to facilitate the differentiation of *C. jejuni* from *C. coli*. *Campylobacter* isolates were confirmed to be *C. jejuni* or *C. coli* using *MapA* and $Col_{2,3}$ primers as recorded by Manfreda *et al*. (2003). All *C. jejuni* and *C. coli* isolates produced amplified fragment at 589 and 462 bp, respectively (El-Jakee *et al*., 2008).

Although, *Campylobacter* is one of the most common bacterial causes of gastroenteritis worldwide very little is still known about the virulence factors of this enteropathogen. *flaA* and *cadF* genes involved in adhesion and colonization of the host's intestine (Nuijten *et al*., 1992; Konkel *et al*., 1999); *ceuE* gene encodes a binding-protein transport system for the siderophore enterochelin (Gonzalez *et al*., 1997), the Cytolethal Distending Toxin (CDT) is responsible for the holotoxin binding to cell membrane (Lara-Tejero and Galan, 2001).

The aim of this study was to estimate the prevalence of virulence (*cadF*, *flaA*, *ceuE*) and CDT toxin encoding genes (*cdtA*, *cdtB, cdtC*) among the isolates and compare the genetic differences between *C. jejuni* and *C. coli* isolated from apparently healthy and diarrheic chicken. The product of *ceuE* gene is important for pathogenicity because of its involvement in iron acquisition and bacterial infectivity (Ketley, 1997). It is clear that 100% of *C. jejuni* and *C. coli* isolates (each) had *ceuE* gene. Talukder *et al.* (2008) found a prevalence of 82.5% in clinical isolates of *C. jejuni*.

One of the best characterized *Campylobacter* virulence markers is the *flaA* gene which determines the flagella formation, hence bacteria motility and enterocyte colonization (Nuijten *et al*., 2000). Results of the present study showed that 35.7 and 83.3% of *C. jejuni* and *C. coli* isolates, respectively possessed the *flaA* sequence. In fact, the role of motility in the invasion of host cells was demonstrated, since the flagellated *C. jejuni* mutants show markedly reduced internalization into the host cells in vitro (Wassenaar and Blaser, 1999).

The *Campylobacter* adhesion to fibronectin (*CadF*) outer membrane protein was shown to be important for full binding capacity of *C. jejuni* to chicken epithelial cells (Flanagan *et al*., 2009). The mutants in the genes *cadF* and *pldA*, the structural gene for phospholipase A, are impaired in their ability to colonize the cecum, indicating that these genes may play a prominent role in successful colonization (Ziprin *et al*., 2001). The *cadF* gene was presented in 78.6 and 83.3% of the examined *C. jejuni* and *C. coli* isolates. Other authors also identified this virulence gene in all or almost all campylobacters tested that were derived from poultry carcass and feces and from human clinical specimens (Datta *et al*., 2003; Rozynek *et al*., 2005; Wieczorek and Osek, 2011).

Cytolethal Distending Toxin (CDT) that comprised three subunits (*cdtA*, *cdtB* and *cdtC*), causes cytodistention of affected epithelial cells and arrest of cell cycle resulting in apoptosis (Lara-Tejero and Galan, 2001).

With respect to cdt, our results confirm the high prevalence of the *cdtA*, *cdtB* and *cdtC* genes in *C. jejuni* (50%), interestingly, all *C. coli* isolates did not carry *cdtA* and *cdtC* genes. All three cdt genes (*cdtA*, *cdtB* and *cdtC*) have been reported to find in nearly all *C. jejuni* strains isolated from both children and chickens (Rozynek *et al*., 2005).

The frequency of a plasmid virulence factor pVir was higher (53%) in patients with bloody diarrhea in comparison to patients with non-bloody diarrhea (21%), whereas other clinical parameters did not differ significantly (Tracz *et al*., 2005). It is clear that 71.4 and 33.3% of *C. jejuni C. coli* isolates respectively carried one or more plasmids.

Schmidt-Ott *et al*. (2005) identified homologues of a type IV secretory apparatus on a large plasmid in only a few isolates, whereas the majority harboured plasmids that belong to a subgroup distinct from pVir.

Campylobacter associated gastroenteritis is normally self-limiting and antimicrobial treatment is reserved for patients with severe and advanced infections (Kurincic *et al*., 2005). *Campylobacter* has developed resistance to several antimicrobial agents over the years, including macrolides, quinolones and fluoroquinolones, becoming a significant public health hazard (Di Giannatale *et al*., 2014).

The present data illustrated that the *C. jejuni* and *C. coli* isolates have a multidrug resistance against many Antimicrobials which may be of public health concern.

CONCLUSION

In conclusion, findings from this study have established that the PCR used in this study allowed showing possible variations present in the expression of so-called classical virulence factors. The molecular characterization of *Campylobacter* demonstrated that, the presence of the *cdtabc* operon, especially in *C. jejuni*, confirm this species as the major pathogen for humans. Present findings indicate the potential danger of antibiotic resistant *Campylobacter* species which could subsequently be transferred to humans through chicken. Further studies appear necessary to evaluate antibiotic resistance of *Campylobacter* isolated from human and animal samples in order to control the emergence of new multidrug resistant strains in Egypt.

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