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Antimicrobial Resistance and Virulence Genes of *Campylobacter jejuni* Isolates from Diarrheic Sheep

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ABSTRACT

One of the important agents causing gastroenteritis worldwide is *Campylobacter jejuni (C. jejuni)*. The current study aimed to detect five virulence genes (flaA, virB11, ciaB, iam, and dnaJ) and two antibiotic resistance genes (gyrA and tetO) in *C. jejuni* obtained from sheep stool. The virulence genes were detected by PCR in 64 *C. jejuni* strains. The phenotypic resistance to five selected antibiotics (Ciprofloxacin, Erythromycin, Gentamycin, Streptomycin, and Tetracycline) was screened with the microdilution method. The isolates with antibiograms were tested for detection of *gyrA and tetO* genes via PCR using specific primers. The virulence genes *flaA* (32%) and *dnaJ* (29%) had the highest prevalence. The tested isolates of *C. jejuni* revealed high resistance to both quinolone (68.3%) and tetracycline groups (48.4%) with an increased prevalence of antibiotic resistance of gyrA and tetO genes. Gentamycin and erythromycin offered better alternative drugs for the treatment of campylobacteriosis. To generalize the findings, extensive profiling that involves more virulence genes is required in several strains of *Campylobacter*.

Keywords: Antibiotic resistance, *Campylobacter jejuni*, Sheep, Virulence genes

INTRODUCTION

Campylobacter is a zoonotic infection that causes foodborne diarrhea in people all over the world (Sheppard and Maiden, 2015; Babazadeh and Ranjbar, 2022). The most common *Campylobacter* species that cause different infections in sheep, including enteritis, colitis, and reproductive disorders, are *Campylobacter jejuni* (*C. jejuni*), *Campylobacter fetus* subspecies *fetus*, and *Campylobacter coli* (*C. coli*, İlhan et al., 2021). Sheep breeding represents a large and essential part of animal husbandry in Egypt, and consequently, diarrhea caused by *Campylobacter* species can influence the production characteristics in Egypt.

Campylobacter species are globally found in soil, water, and food, so they can be in the gall bladder and the intestine without any clinical signs as a result of contact with contaminated sources, such as animal stool, genital excretions, and aborted tissues (Indykiewicz et al., 2021). Wild and domestic animals have been identified as potential carriers of this bacteria (Rukambile et al., 2019). *Campylobacter* species are present in the gut of many animal species with interspecies transmission risk. *Campylobacter* species, such as *C. jejuni* and *C. coli*, can be isolated from different farm animals, including cattle, sheep, and goats. Comparing sheep and goat breeding farms, sheep are more potential carrier of *C. jejuni* as a contaminant (Pao, et al., 2014).

Healthy sheep act as reservoirs where the bacteria are intermittently excreted in their feces, particularly in stressful situations (birth, weaning, and changes in feeding systems) although their activity is rapidly inactivated on pastureland at high temperatures. It is worth noting that the presence of wild birds in sheep farms raises the potentiality of lamb infection, especially at very young ages (Sproston et al., 2010).

The gene expression for motility, colonization, invasion, and excretion of toxins is believed to be an essential cause of disease progression (Dasti et al., 2010). Bacterial cell movement involving the coordination of many genes (such as *flaA*) is responsible for the bacteria passage through the gastrointestinal environment (Park, 2002) where *Campylobacter* delivers many cell surface proteins encoded by several genes (such as *virB11, ciaB,* and *iam*) that support adhesion and invasion of enterocytes (Dasti, et al., 2010). Furthermore, *C. jejuni* can produce defense factors such as cytokines and enzymes like superoxide dismutase to get rid of superoxide radicals as a defense mechanism against oxidative damage.

Campylobacteriosis is considered a self-limiting disease. In acute cases, macrolides Macrolides, fluoroquinolones, and aminoglycosides are classified as critically important antimicrobials, while tetracycline is considered a highly important antimicrobial (World Health Organisation, 2018). Unfortunately, nowadays there is a growing trend of antibiotic resistance among *Campylobacter* species mainly due to misuse of antibiotics (Wieczorek et al., 2017). Therefore, the present study was designed to find the prevalence of virulence and antimicrobial resistance genes of *C. jejuni* isolated from sheep suffering from diarrhea.

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MATERIALS AND METHODS

Ethical approval

Ethical approval was not necessary for this study; however, samples were collected as per standard sample collection procedure and consent was taken from the animal owners with their signature using a prescribed consent form (License No. AHRI 42102017), according to local Egyptian laws.

Collection of *Campylobacter jejuni* isolates

A total of 262 fecal samples were collected from sheep aged 2-3 years (Barki sheep ewes and Rams) suffering from different levels of watery diarrhea. The clinical signs included diarrhea, decreased appetite, and vomiting with or without fever. The feces were usually watery or bile streaked with mucus and sometimes blood. The animals were obtained from different sheep herds on the Northwest coast of Egypt. All collected samples were transferred in sterile plastic bags and refrigerated up to the time of investigation (within 24 hours after collection). *Campylobacter* isolation was performed by the culture method following a study by Hagos et al. (2021). The strains were grown on blood-based agar (BD BBLTM, United States) with 5% defibrinated sheep blood and incubated at 42°C for 48 hours under microaerobic conditions (85% nitrogen, 10% carbon dioxide, and 5% oxygen). The strains were confirmed as *C. jejuni* or *C. coli* using Lior's biotyping scheme (Lior, 1984) and the PCR technique based on the highly conserved gene glyA (serine hydroxymethyltransferase, Quino et al., 2022).

DNA extraction and PCR

All *Campylobacter* isolates were subjected to DNA extraction following the instructions of QIAamp DNA Mini kit (Qiagen, Germany, Catalogue no.51504) with slight modifications. Briefly, 10 µl of proteinase K and 200 µl of lysis buffer were added to 200 µl of the sample DNA and incubated at 56°C for 10 minutes. Then, 200 µl of absolute ethyl alcohol was added to the lysate. The sample was washed and centrifuged. Nucleic acid was obtained in 100 µl of elution buffer. Then, the PCR technique was carried out for thermophilic *Campylobacter* species (*C. jejuni* and *C. coli*, Iraola et al., 2012).

Investigation of virulence genes

Campylobacter isolates were examined for the virulence genes of *flaA* (responsible for motility) and *virB11* (for adhesion and colonization). In Addition, the gene markers of *ciaB* and *iam* (for the *Campylobacter* invasiveness) were also amplified. The primer sets targeting the 23S rRNA gene of *Campylobacter* species, and the virulence genes of *flaA, dnaJ, virBII, iam,* and *ciaB* were used and specific amplified products were detected at 217, 177, 494, 518, and 527 bp, respectively. The amplicons were detected using capillary electrophoresis. Primer sequences, target genes, amplicon sizes, and cycling conditions are illustrated in Table 1. PCR conditions and techniques for all the above genes were based on a study by Datta et al. (2003).

Table 1. PCR primers used for *Campylobacter* detection and antimicrobial resistance genes

Target genes	Primers sequences	Amplified segment	Reference
23S Rrna	TATACCGGTAAGGAGTGCTGGAG ATCAATTAACCTTCGAGCACCG	650	Wang et al. (2008)
FlaA	TCCAAATCGGCGCAAGTTCA TCAGCCAAAGCTCCAAGTCC	217	Zheng et al. (2006)
DnaJ	ATTGATTTTGCTGCGGGTAG ATCCGCAAAAGCTTCAAAAA	177	Chansiripornchai and Sasipreeyajan (2009)
virB11	TCTTGTGAGTTGCCTTACCCCTTTT CCTGCGTGTCCTGTGTTATTTACCC	494	Datta et al. (2003)
Iam	GCGCAAATATTATCACCC TTCACGACTACTACTATGCGG	518	Wieczorek, (2011)
ciaB	TGC GAG ATT TTT CGA GAA TG TGC CCG CCT TAG AAC TTA CA	527	Zheng et al. (2006)
tetO	GGCGTTTTGTTTATGTGCG ATGGACAACCCGACAGAAGC	559	Gibreel et al. (2004)
gyrA	GATGGTTTAAAGCCTGTTCAT CGCCATACCTACAGCTATACC	423	Lindmark et al. (2004)

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Antimicrobial resistance

Campylobacter jejuni isolates were evaluated for the resistance to selected antimicrobial agents with the microdilution method using microtitration plates. The five tested antimicrobial drugs are the most common ones used in the treatment of *Campylobacter* infections. Solutions of each tested antibiotic included streptomycin, ciprofloxacin, tetracycline, erythromycin, and gentamicin solutions (Table 2). Mueller Hinton broth with 2.5% lysed horse blood was prepared. The inoculated plates were incubated at 37°C for 48 hours in a microaerophilic atmosphere (the same conditions as above). The parameters for individual antibiotics, including interpretation criteria, were based on recommendations issued by the CLSI guidelines (McDermott et al., 2005). Quality control was done using a reference strain of *C. jejuni* (ATCC 33560). The detailed parameters for testing are shown in Table 2.

Investigation of antibiotic resistance genes

Once the identification was performed, the isolates were screened for the existence of resistance genes to quinolone, the Thr-86-lle mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* gene in *C. jejuni* and the tetracycline resistance gene (*tetO)* were amplified and identified using PCR with two specific primer sets for amplification at 423 and 559 bp.

Sequence

PCR products were purified using QIAquick PCR Product extraction kit (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction which was then purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 Genetic Analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) was initially performed to establish sequence identity to GenBank accessions (Altschul et al., 1990). The phylogenetic tree was created by the MegAlign module of LasergeneDNAStar version 12.1 (Thompson et al., 1994), and Phylogenetic analyses were done using maximum likelihood, neighbor-joining, and maximum parsimony in MEGA6 (Tamura et al., 2013).

RESULTS

Investigation of *Campylobacter jejuni* spacemen

A total of 262 samples from sheep feces (235 diarrhea and 27 non-diarrhea) yielded 64 *C. jejuni* strains (24.4%). Two *C. jejuni* strains were found in stool samples that appeared to be non-diarrheal, and 62 *C. jejuni* strains were found in clearly diarrheal stool samples.

Detection of antimicrobial susceptibility of *Campylobacter jejuni* isolates

Of the 5 antibiotics tested, the highest phenotypic resistance exhibited by *C. jejuni* from stool samples was against ciprofloxacin (68.3%), followed by tetracycline, streptomycin, gentamycin, and erythromycin as 48%, 4%, 27.5%, 6.3%, and 4.5%, respectively while complete susceptibility (100%) was detected against gentamycin (Table 2). The highest prevalence of resistant strains was against type 4 of antibiotics (24%) while resistance to type 5 was the least (5%, Figure 1).

Investigation of antibiotic resistance genes in *Campylobacter jejuni*

Following the detection of phenotypic resistance to the chosen antibiotics, the resistance of *gyrA and tetO* genes was detected using PCR. The *tetO* gene which is liable for tetracycline-resistant was detected at 595 bp while the *gyrA* gene which is responsible for the quinolone resistance was detected at 423 bp (Figure 2). A comparison of the presence of selected antibiotic resistance genes with the phenotypic resistance is shown in Table 3. As can be seen, the resistance in the evaluated genes is more common in isolates that showed phenotypical resistance.

Detection of virulence genes in *Campylobacter jejuni* isolates

A total of 24.4% of examined stool samples contained *C. jejuni*. The proportion of the virulence genes in *C. jejuni* isolates is displayed in Figure 3. The results revealed that the *flaA* gene (93%), which encodes the motility, is the most prevalent virulence gene in *C. jejuni* isolates followed by *dnaJ* encodes heat shock protein (ATPase activity), and *ciaB*; encodes invasion in 88% and 42%, respectively. Two genes of *virBII* and *iam* recorded the minimal frequency as 7.3% and 6.8%, respectively.

Investigation of phylogenetic relationship

Based on the analysis of 23S rRNA, dendrograms help to determine simmilarites and difference between the isolates when compared to the reference strains (Figure 5). The graphs illustrate that *C. jejuni* isolates differ significantly. According to present dendrogram, the 5 analyzed isolates (3, 4, 6, 7, and 11) are characterized by genetic variation (Figure 6). Strains 3, 4, and 6 are closely linked with a smaller degree of relatedness to strain 11, however, strain 7 has a lot of genetic diversity, compared to the other strains. OK095294, OK095295, OK095296, OK095297, and OK095298 are the accession numbers of isolates 3, 4, 6, 7, and 11, respectively. However, all strains were similar to *C. jejuni* strains present in gene bank by 98.3-100% (Figure 6).

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Table 2. Profiles of antimicrobial-resistant *Campylobacter jejuni* for different antibiotics

Antibiotic	Antibiotic dilution (mg/L)	*C.jejuni* dilution (mg/L)	Resistance in fecal isolates (%)
Ciprofloxacin	0.03-64	0.5	68.3
Tetracycline	0.125-256	1	48.4
Streptomycin	0.25-512	4	27.5
Gentamycin	0.125-256	2	6.3
Erythromycin	0.25-512	4	4.5

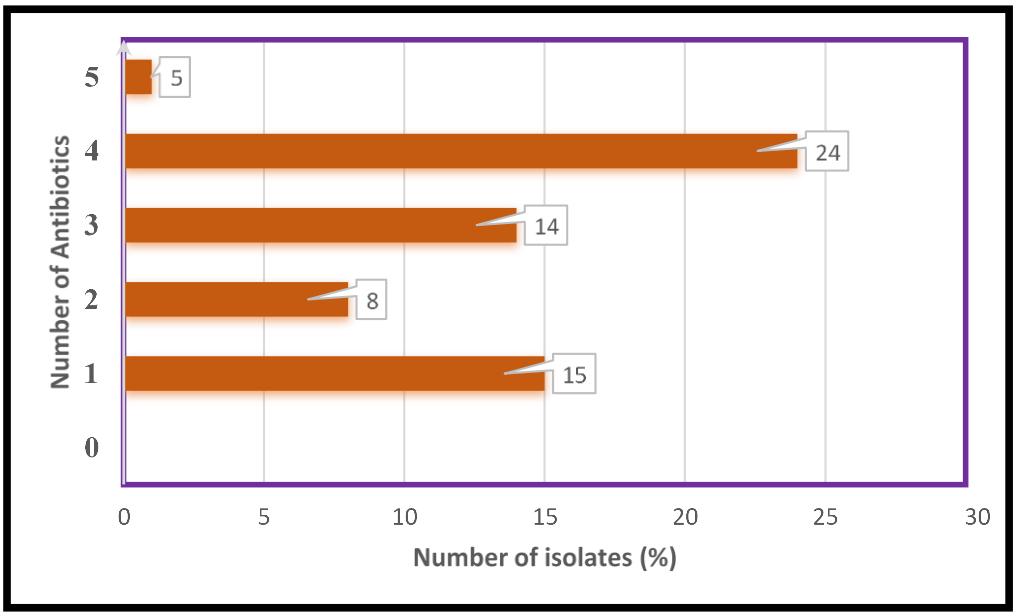


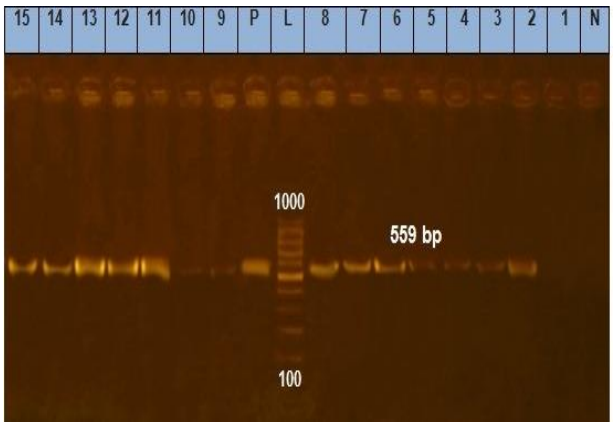
Figure 1. *Campylobacter jejuni* with antimicrobial resistance

Table 3. Relationship between genotypic and phenotypic resistance to ciprofloxacin and tetracycline in *Campylobacter jejuni*

Detection of the *gyrA* gene in *Campylobacter jejuni* isolates (%)		Detection of the *tetO* gene in *Campylobacter jejuni* isolates (%)	
Cipro-R	Cipro-S	Tet-R	Tet-S
78.3	35.5	43.6	21.7

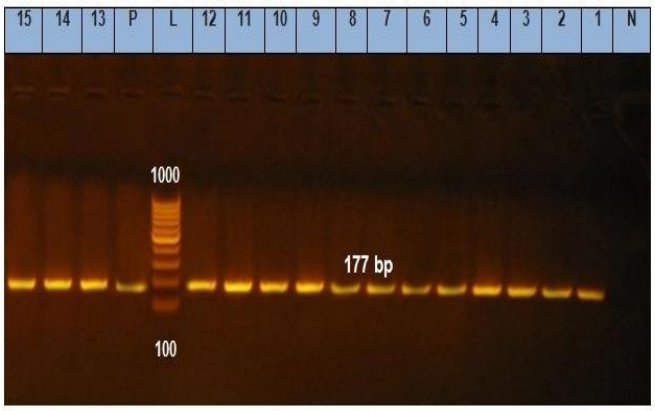
Cipro-R: Isolate with phenotypic resistance to ciprofloxacin. Cipro-S: Isolate with phenotypic susceptibility to ciprofloxacin. Tet-R: Isolate with phenotypic resistance to tetracycline. Tet-S: Isolate with phenotypic susceptibility to tetracycline

A



***tetO* gene** samples were positive at 559bp except sample at lane 1

B



***gyrA* gene all** samples were positive at 177bp

Figure 2. Genotypic characterization of antimicrobial resistance in *Campylobacter jejuni*. **A:** Detection of *tetO* gene, **B:** Detection of *gyrA* gene.

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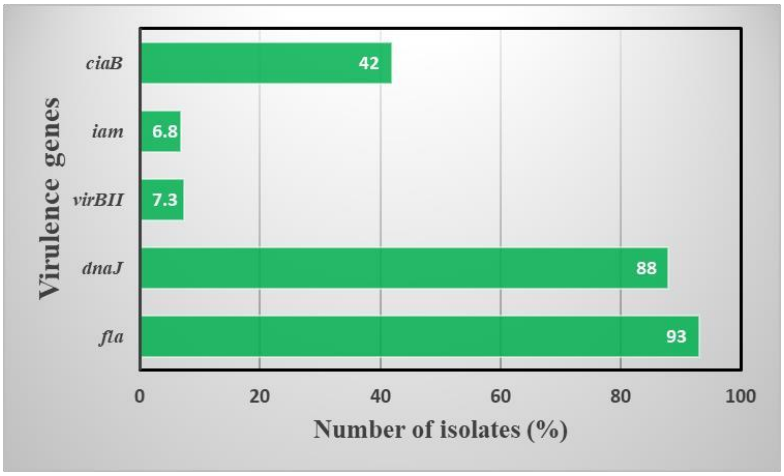
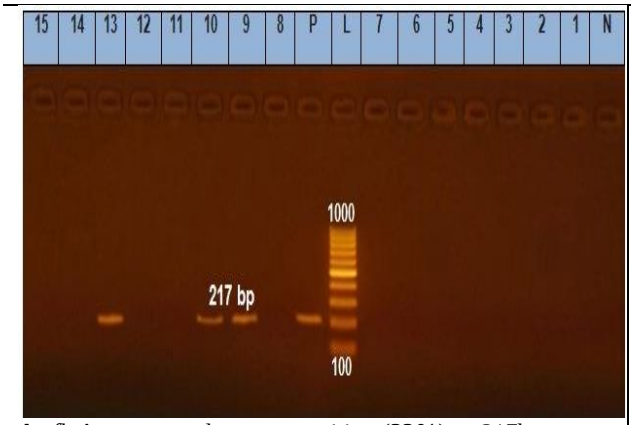
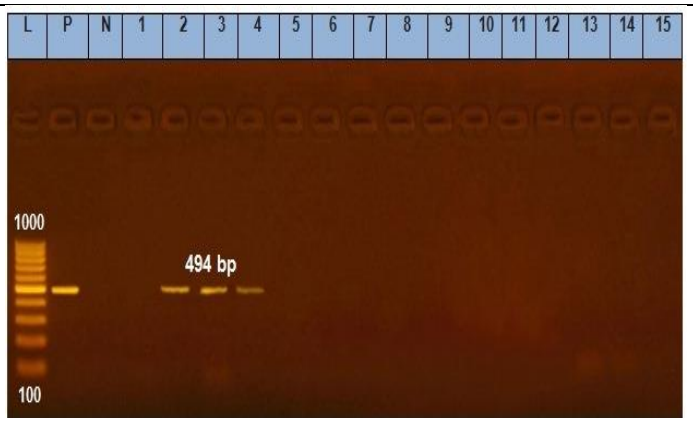


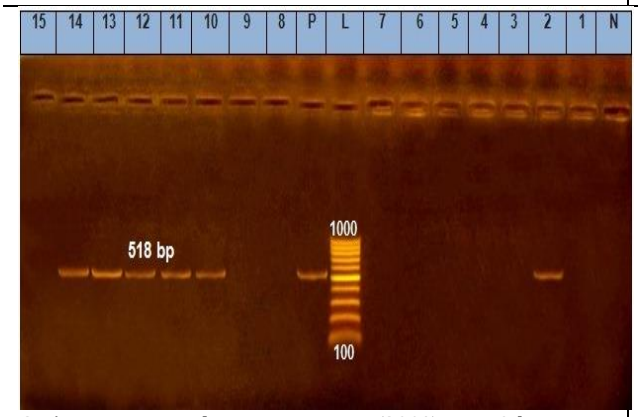
Figure 3. Prevalence of virulence genes in *Campylobacter jejuni* from Barki sheep stool



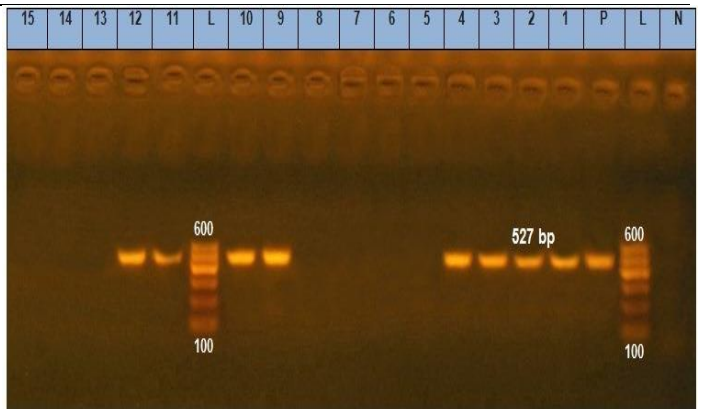
A: *flaA gene* samples were positive (**32%**) at 217bp except sample at lanes 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 14, and 15



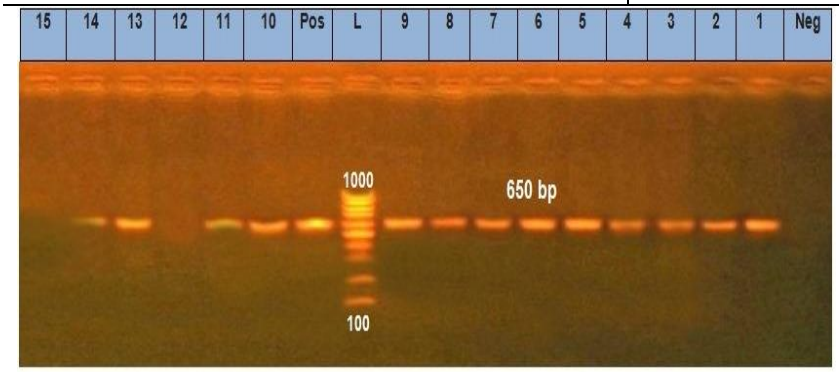
B: *virBII gene* samples were positive (**15%)** at 494 bp except sample at lanes 1, , 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15



C: *iam gene* samples were positive (**29%**) at 518 bp except sample at lanes 1, 3, 4, 5, 6, 7, 8, 9, and 15



D: *ciaB gene* samples were positive (**27%**) at 518 bp except sample at lanes 5, 6, 7, 8, 13, 14, and 15



E: *23S rRNA* samples were positive at 650 bp except sample at lanes 12 and 15

Figure 4. Detection of virulence genes and 23S rRNA of *Campylobacter jejuni* isolates from Barki sheep stool. **A:** Detection of *flaA* gene. **B:** Detection of *virBII*. **C:** Detection of *iam* gene. **D:** Detection of *ciaB* gene. **E:** Detection of 23S rRNA

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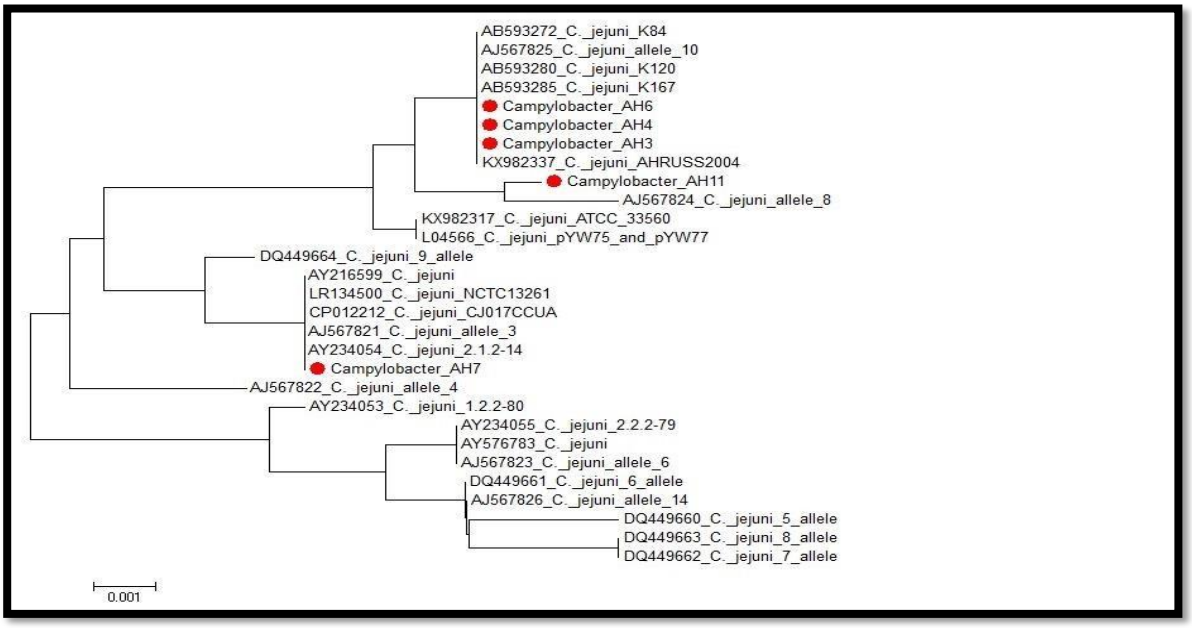

Figure 5. *Campylobacter jejuni* phylogenetic relationship

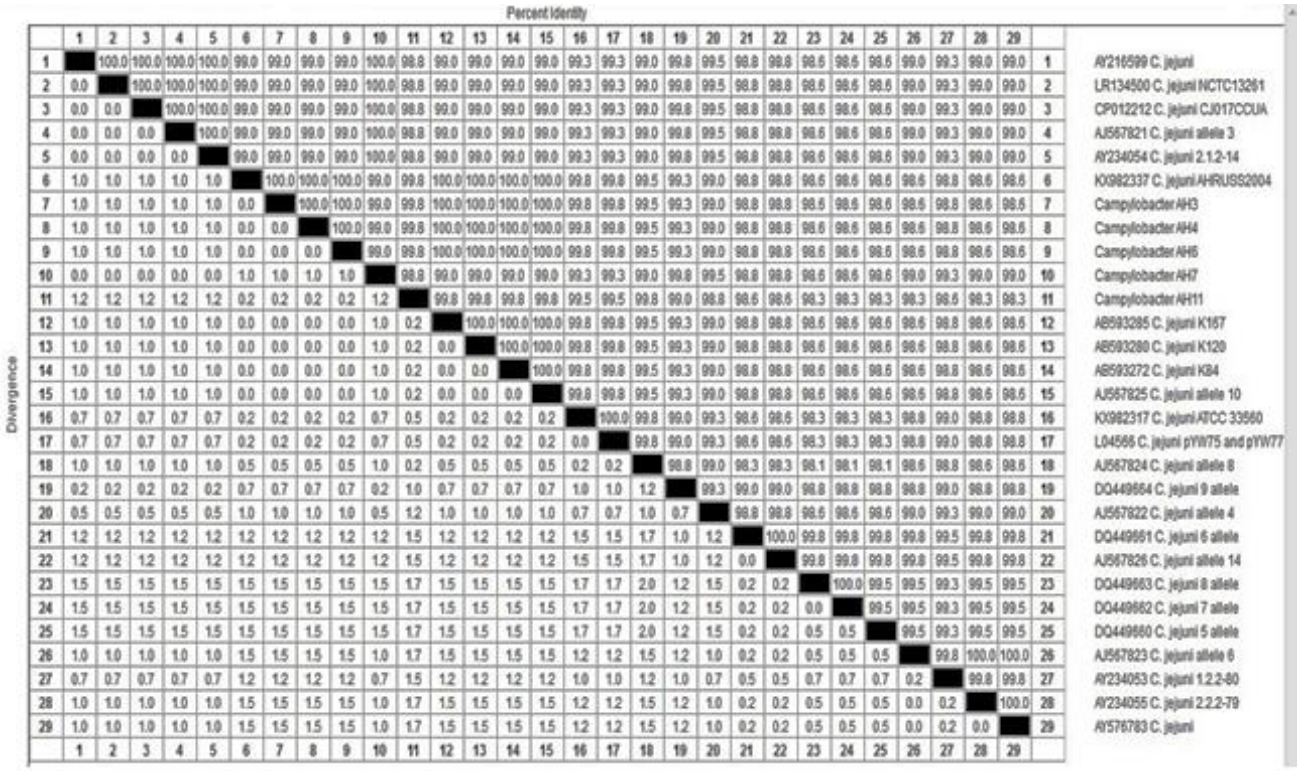

Figure 6. Sequence distance of 5 *Campylobacter jejuni* isolates from Egypt, compared with 24 *Campylobacter jejuni* in the gene bank

DISCUSSION

The intermittent nature of *Campylobacter* infection explains why there are different reports on this type of infection and impeding the discovery of its source (Havelaar et al., 2013). It is established that poultry is the main source of human infection (Ranjbar and Babazadeh, 2017; Nur-Aziera-Aina et al., 2020), *Campylobacter* spp. is also highly prevalent in ruminants all over the world (Babazadeh and Ranjbar, 2022). There is growing data that the ruminants play a pivotal role in the spreading campylobacteriosis to humans as cattle and sheep are considered the second most important reservoir after broiler for the transmission of *C. jejuni* infection to humans (Roux et al., 2013).

Of 302 fecal samples from sheep in Shiraz, Iran, 67.8% were positive for the presence of *Campylobacter* species isolates showed high resistance to cephalothin (83%) and ciprofloxacin (67.7%, Khoshbakht et al., 2016). Regarding the

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obtained results of the current study, there was no antibiotic that could trigger the sensitivity of all *Campylobacter* isolates. Fluoroquinolones are one of the recommended drugs for campylobacteriosis treatment. The *Campylobacter* strains resistant to ciprofloxacin were established in the late 1980s, indicating that the animals play a key role in generating and transmitting the resistant bacteria. At Present, *C. jejuni* resistance to fluoroquinolones is increasing worldwide which poses a threat to public health (Wieczorek, 2011). The current findings indicated that the treatment with fluoroquinolones has become inefficient as some *Campylobacter* strains are resistant to this class of antibiotics (Bolinger and Kathariou, 2017).

Antibiotic resistance was high against the ciprofloxacin (68.3%), and this is consistent with a previous prevalence report in South Africa where rates of *Campylobacter* resistance to fluoroquinolones have been reported between 14.8% and 51.3% (Kepner et al., 2003). These results show that *Campylobacter* resistance to fluoroquinolones can increase over the years. Therefore, constant monitoring is necessary as *Campylobacter* species can mutate (Luo et al., 2003).

High tetracycline resistance has been recognized globally. Modifications in media to test *C. jejuni* isolates from several countries in the European Union indicated a resistance of about 45% (Aleksić at al., 2021). In the present study, there was relatively higher tetracycline resistance (48.4%). The higher resistance of *C. jejuni* to the tetracycline group may be due to the overuse of this group of antibiotics as they are given to treat most infections in the veterinary field in Egypt (Schiaffino et al., 2018). The comparatively high co-resistance of some strains of *C. jejuni* to tetracycline and/or ciprofloxacin is also important due to their clinical significance in the treatment of severe cases of campylobacteriosis. Therefore, the best solution is to use other groups of antibiotics, such as aminoglycosides and macrolides, and to use them only in severe cases where mild forms of *Campylobacter* should be considered a self-limiting infection.

In the current study, *C. jejuni* strains from sheep stool were examined for resistance to ciprofloxacin. An increase in *C. jejuni* resistance to ciprofloxacin was detected (68.3%), which was similar to previous studies in Poland (Wieczorek and Osek, 2013) and other EU countries (EFSA, 2014a; EFSA, 2014b). It must be taken into consideration that the prevalence of resistance can change significantly over time ($p < 0.05$), amino acids substitution is the main cause of fluoroquinolones resistance in *Campylobacter* (Wieczorek and Osek, 2013). The most common silent mutations in the quinolone resistance determination region of gyrA are presented in Table 3. Thr86Ile substitution in the gyrase reveals high-level resistance to this antibiotic group (Payot et al., 2006). In agreement and confirmation of this and other similar studies (Duarte et al., 2014), the Thr86Ile substitution was the most detected amino acid change. On the other hand, further mechanisms of resistance, such as alteration in the outer membrane permeability and efflux systems, have been reported (Charvalos et al., 1996) and these may explain the detection of phenotypic resistance without amino acid changes in *gyrA* in the tested strains.

It is noteworthy that the silent mutation in *Campylobacter* species was reported in both resistant and sensitive strains to ciprofloxacin and a high number of combinations of transitions and mutations may exist. The current work confirmed these results, and some silent mutations that are frequently observed at Ser-119 → His, Glu-131 → Glu, and Ser-157 → Ser correspond to mutations detected in *Campylobacter* strains isolated in Finland and Brazil (Hakanen et al., 2002).

In the present study, gentamycin and erythromycin exhibited a lower resistance at 6.3% and 4.5%, respectively. Therefore, they offered a better alternative drug for the treatment of campylobacteriosis. It is interesting to note that when using macrolides (erythromycin) in treatment, attention should be devoted to testing resistance to erythromycin.

The mechanism through which the *Campylobacter* species cause enteritis is a complex process depending on many factors where specific genes are implicated in all virulence stages of adhesion, colonization, invasion, and toxin production (Bolton, 2015). To evaluate the pathogenicity of the *Campylobacter* isolates in the present study, the existence of five essential genes coding the virulence factors, such as the motility (*flaA*), invasive (*iam* and *ciaB*), ATPase activity (*dnaJ*), and adhesion (*virBII*) genes in the isolates have been investigated.

The first step in pathogenesis is intestinal colonization. This requires the motility of the microbe into the mucus layer that covers the enterocytes. *Campylobacter* motility is granted by the polar flagella in 'cork-screw' shape movement allowing them to effectively penetrate and overcome this mucus barrier (Haag et al., 2012). The flagellin protein encoded by the *flaA* gene is considered the most virulence factor that has been studied and characterized in *Campylobacter* species (Hermans et al., 2011).

The higher prevalence of *flaA* gene (93%) among the *Campylobacter* isolates in the present study is nearly consistent with an Egyptian study by Abd El-Hamid et al. (2019), where the *flaA* gene was detected in all isolates (100%). On the other hand, the *flaA* gene prevalence was inconsistent with other published studies where the prevalence was 87.5%. This discrepancy can be attributed to the identification of a higher number of virulence genes.

The second step in pathogenesis is adhesion. Gene liable for the adhesion of *C. jejuni* is *virBII* that is responsible for producing the IV secretory system protein and is located on the pVir plasmid. It has been reported that strains that show a mutation in the *virB11* sequence have a much lower ability in adhesion and penetration when compared with the original strains, and hence, lower pathogenicity (Bacon et al., 2000). In the present study, the gene was detected in 7.3% of the isolates, which was in accordance with the previous work with a prevalence rate of 9.7% (Abd El-Hamid et al.,

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2019). One of the most crucial genes for adhesion and invasion is the *ciaB* gene *(Campylobacter* invasive antigen B). The *ciaB* gene has been reported to be involved in the invasion of the enterocytes and plays a significant role in colonization (Ó Cróinín and Backert, 2012). and was detected in 42% of the tested strains. The low frequency of the *ciaB* gene in the clinical isolates disagrees with other previously published data reporting 100% detection of this virulence gene in their isolates (Biswas et al., 2011). Therefore, the results obtained from the current study confirm the claim that not all *Campylobacter* strains having the *ciaB* gene can adhere to or invade enterocytes. This indicates the presence of other factors than those coded by the *ciaB* gene on *Campylobacter* surfaces, and this perception is confirmed by previous studies (Bolton, 2015). In accordance with the current study, a lower prevalence of *ciaB* (76.4%) has been measured in Qatar, while the prevalence range of 52.4-71.4% was reported in Asia and 51.5-66.7% in the Arabian Peninsula (Carvalho et al., 2001).

Another invasion-associated marker (*iam*) gene is one of the most essential factors for the invasion of the host cell and was detected in the current study with a prevalence of 6.8%. This prevalence is considered too low, compared to earlier studies where the prevalence reaches 85%. This divergence may be due to the scarcity of *iam* in the isolated *Campylobacter* strains and therefore, its role in the *Campylobacter* pathogenesis should be further assessed (Wieczorek et al., 2018).

The bacterial response to the thermal stress is mainly via the expression of heat shock proteins. These proteins play an important role in thermotolerance. They act as chaperones to improve the folding of cellular protein, and degradation of possibly deleterious misfolded proteins. Several heat shock proteins were identified in the *C. jejuni*, including *DnaJ, DnaK, GroESL,* and *ClpB* genes. However, the most important one is *dnaJ* gene, as any mutation in *C. jejuni* unable the bacteria to colonize the enterocytes (Konkel et al., 1998). In the present work, *dnaJ* gene was detected in 88% of all tested sheep fecal samples. Relatively similar results were reported by many authors (Redondo et al., 2019) who verified the importance of *dnaJ* gene for colonization.

CONCLUSION

Campylobacteriosis control and prevention in sheep requires an understanding of the transmission routes, antibiogram, and virulence abilities of the isolates. The results gained in the current study demonstrated the presence of *Campylobacter* isolates and different degrees of resistance. The prevalence of the resistance may mainly be attributed to the misuse of antibiotics used for the treatment of *Campylobacter* infections, such as ciprofloxacin and tetracycline. Although the isolated strains carried both virulence and antibiotic resistance genes, continuous monitoring of the prevalence of *Campylobacter* strains and identification of associated genes for virulence and antibiotic resistance is urgently required to update effective treatment schedules for *Campylobacter* infection.

Finally, it is worth mentioning that the presence of virulence genes is an important predictor of strain virulence although it may not exactly predict the virulence of the isolated *Campylobacter* strains. Additionally, negative PCR results do not mean that there is no gene but could be attributed to a different primer binding site sequence or the presence of another gene with a similar function. To generalize the findings, extensive profiling that includes more virulence genes is required for other strains of *Campylobacter*.

DECLARATIONS

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Author's contribution

Amani Hafez performs collection, preparation, processing, and analysis of samples, isolation of bacteria, data acquisition, writing, preparation, and revision of the manuscript. The author has read and approved the data and final draft of the manuscript.

Competing interests

The author has declared no conflict of interest.

Ethical consideration

The author checked the manuscript for ethical issues, such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publishing and/or submission, and redundancy.

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