Genotypic Characterization of *Campylobacter* Species Isolated from Livestock and Poultry and Evaluation of some Herbal Oil Antimicrobial Effect against Selected *Campylobacter* Species

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Abstract | Campylobacter food poisoning is underestimated in developing countries. This study aimed to investigate the prevalence and molecularly characterize *Campylobacter* spp. in cattle, sheep and poultry investigated in Beni-Suef Governorate, Egypt. Additionally, the MICs of some selected herbal oils on the isolated strains were studied. A total of 190 rectal swabs from cattle (n=85) and sheep (n=105) in addition to 200 samples from chickens (70 intestinal content and 130 cloacal swabs) were collected in the period October 2016 through January 2017. Bacteriological examination revealed that 37 (43.52%) out of 85 rectal samples obtained from cattle, as well as 37 out of 105 sheep samples (35.24%) harbored *Campylobacter* spp. In addition, 146 (73%) out of 200 examined chicken samples were bacteriologically positive. Analysis of the identified *Campylobacter* spp. revealed that the *C. coli* was more prevalent in cattle and sheep than *C. jejuni* (13.5 and 21.6%, respectively). In chickens, results showed also that *C. coli* was found in 15% of the tested samples, while *C. jejuni* failed detection. The results showed high prevalence rates of virulence genes in tested strains. The flaA gene as a *Campylobacter* pathogenic marker was detected in (100%) of analyzed strains. The Cdt toxin three subunits: CdtA, CdtB, and CdtC were also detected in all tested strains. Twenty highly virulent *Campylobacter* strains (14 *C. coli* and 6 *C. jejuni*) were exposed to herbal oils in order to determine the MIC. The results showed that MIC values of selected herbal oils against *Campylobacter* spp. were 500, 1000, 1500, and 2000 µg/ml for eugenol, cinnamon, allicin, and thyme, respectively. The studied essential oils appeared to be effective against the highly virulent local *Campylobacter* strains at low bactericidal concentrations, thus, emphasizing the significance of these oils as natural antimicrobial agents.

Keywords | *Campylobacter* spp., Virulence factors, Livestock, Poultry, Herbal oils

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INTRODUCTION

*Campylobacter* is the main zoonotic pathogen that causes foodborne enteritis with *C. jejuni* and *C. coli* being the most recovered species (Scallan et al., 2011). *Campylobacter* infections in humans are mainly associated with consumption of undercooked chickens, improperly cooked beef, pork and raw milk. Exposure to farm animals and untreated water are other transmission routes. The significant role of cattle and sheep as important reservoirs of *Campylobacter* has been proven through molecular epidemiological research (Kassa et al., 2006; Sahin et al., 2012). *C. jejuni* and *C. coli* are commonly carried as commensals in the intestine of these animals, thus posing a considerable problem for the disease controller. Frequent screening of these animals for fecal carriage is, therefore,
an important step for avoiding spread of these pathogens to humans via the fecal-oral route.

Poultry, particularly broiler chickens, are as important as ruminants in the epidemiology of campylobacteriosis. Sources of infection for poultry include water, feed, and litter. The birds usually do not show any signs of disease, but bacteria from the intestines can contaminate carcass surfaces during evisceration in the slaughterhouse and subsequently may transmit to humans (Naglić et al., 2005). In fresh retail poultry products, *Campylobacter* occurs as a result of their colonization of the gastrointestinal tract of chickens during growth.

Chickens play a significant role as a source of human infections in the developed world, hence it was suggested that combating of colonization of *Campylobacter* spp. in avian host can decrease the incidence of campylobacteriosis (Meunier et al., 2016). Decreasing *Campylobacter* colonization of poultry by $2\log_{10}$ was found effective to reduce human infections by 30-fold. Therefore, research has been focused on understanding the colonization of poultry by *Campylobacter*, subsequently, even a small reduction could have an extremely positive impact on human health (Rosenquist et al., 2003).

The mechanism of *Campylobacter* pathogenicity has not been exactly explained yet, although several studies investigated the virulence factors involved in *Campylobacter* pathogenicity (adhesion, invasion, and cytotoxicity) (Khoshbakht et al., 2013). However, the wide genotypic and phenotypic diversity of the bacterial species belonging to the genus of *Campylobacter* made it difficult to conclude the pathogenicity dependent factors.

It was found that some *C. jejuni* strains cause serious illness, while other isolates are not pathogenic at all or induce mild symptoms in humans (Rivera-Amill et al., 2001). However, it is known that mechanisms of movement, chemotaxis, adhesion, transcytosis and host cell penetration, as well as toxin production, are necessary to induce campylobacteriosis in humans (Snelling et al., 2005). These molecular markers participate in adhesion and colonization (*flaA*), invasion (*virB11*), the *wla* gene which is probably involved in the expression of ganglioside mimics in Guillain-Barré syndrome and Cdt cluster (*cdtA, cdtB, cdtC*) which is responsible for toxin production (Wieczorek et al., 2011).

A major problem facing the efforts to control populations of food-borne pathogenic bacteria is the formation of protective biofilms produced by bacterial colonies. *Campylobacter* is 1000 times more resistant to antibiotics due to the presence such phenomenon. However, some essential oils are assumed able to solve such obstacle. For instance, garlic compounds were able to destroy the bacteria in just a fraction of the time taken by antibiotics like, erythromycin and ciprofloxacin (Xiaonan et al., 2012).

Feed manufacturers are targeting many natural antimicrobials from plant material as new kinds of consumer-accepted feed additives to achieve the required purpose (Navarro et al., 2015). Many of them have promising ability to reduce the bacteria both in vitro as well as in foods (Rattanachaikunsopon et al., 2010). In recent years numerous studies have shown that *Campylobacter* species are sensitive to a wide variety of plant-derived compounds such as carvacrol, cinnamaldehyde, eugenol, and thymol (Grill et al., 2012; Navarro et al., 2015).

Eugenol is extracted from many plants, specifically the *S. aromaticum* (clove), and its antimicrobial activity is based on the ability to permeabilize the cell membrane and interact with proteins. Such nonspecific permeabilization of the cytoplasmic membrane is evidenced with leakage of K+ and ATP from the cell by eugenol (Hemaiswarya and Doble, 2009). Another essential oil is cinnamon which has a notable antioxidant activity, especially depending on phenolic and polyphenolic compounds (Aminzare et al., 2015; Moarefian et al., 2013). Allicin, obtained from garlic, contains a wide range of thiolsulphinates that are responsible for the antibacterial activity (Marks, 2014). Garlic contains diallyl sulfide, that effectively penetrates and dissolves the protective bio-layer, and ultimately destroys the bacteria (Xiaonan et al., 2012). In addition, thyme oils induce their antimicrobial activity by triggering structural and functional damages to the cell membrane. Carvacrol causes disintegration of outer membrane and ultimately release of lipopolysaccharides from Gram negative bacteria (La Storia et al., 2011).

The fact that foodborne campylobacteriosis is underestimated in developing countries including Egypt directed us to conduct the current study with the objectives to; determine the prevalence of the *Campylobacter* spp in in cattle, sheep and poultry samples, perform molecular characterization of randomly selected *C. jejuni* and *C. coli* local strains isolated from cattle, sheep, and chickens’ fecal samples targeting seven *Campylobacter* spp. virulence genes, and finally studying the minimum inhibitory concentrations (MICs) of some herbal oils (eugenol,
cinnamon, allicin, and thyme oil) on *Campylobacter* spp. for use to control their populations in foods.

**MATERIALS AND METHODS**

**Samples**

A total of 190 rectal swabs from cattle (n=85) and sheep (n=105) in addition to 200 samples from chickens (70 intestinal content and 130 cloacal swabs) were collected through a 4-monthes period, October 2016 through January 2017. Rectal and cloacal swabs were collected using sterile cotton swabs. Cloacal swabs were collected from poultry just before slaughtering while in case of intestinal contents Ten grams from each sample were taken from the cecal part just after slaughtering through an opening by sterile scissor. All samples were transported to the laboratory in ice box as soon as possible for bacteriological examination.

**ISOLATION AND BIOCHEMICAL EXAMINATION**

The technique was applied as recommended by ISO 10272-1 (ISO, 2006), using Bolton selective enrichment broth (Oxoid, CM0983, UK) with Bolton Supplement (Oxoid, SR0183,UK) and laked horse blood (Oxoid, SR0048,UK), CCDA (charcoal, cefoperazone, desoxycholate agar) medium (Oxoid, CM0739,UK) and CCDA selective supplement (Oxoid, SR0155,UK) and Campy Gen TM microaerophilic sachet (Oxoid,UK) was used to provide microaerophilic conditions required for *Campylobacter* spp. growth.

**IDENTIFICATION, GENOTYING AND VIRULENCE GENES DETECTION IN THE ISOLATED CAMPYLOBACTER SPP.**

Bacterial DNA was extracted using QIAamp DNA mini kit (Qiagen, USA) according to the manufacturer instructions. Oligonucleotide primers (Metabion, Germany) that specifically amplify target *Campylobacter* spp. genes were used for molecular identification (Table 1). The PCR reactions were done in 25µl reactions using Taq DNA Polymerase Kit (Invitrogen, USA) according to the manufacturer instructions with using 20pmol concentration of each forward and reverse primers. The thermal profile consisted of initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing according to each primer pairs for 45 sec, extension at 72°C for 45 sec, and the final extension at 72°C for 10 min.

**EVALUATION OF THE EFFECT OF HERBAL OILS ON CAMPYLOBACTER SPP.**

A total of 20 virulent *Campylobacter* spp. strains (14 *C. coli* and 6 *C. jejuni*) recovered from fecal samples of cattle, sheep and chickens as confirmed by PCR were exposed to herbal oils (eugenol, cinnamon, allicin, and thyme oil 10%) in order to determine the minimum inhibitory concentration (MIC). A bacterial suspension (1-2 x 10^8 CFU/ml, Raeisi et al., 2016) was prepared using physiological saline matching Mcfarland’s opacity tube 0.5 prepared by adding barium chloride to sulfuric acid. The mixture of the two chemicals forms a precipitate, that when in suspension is equivalent to approximately 1.5 x 10^8 colony forming units/ml. (Approximate formula per liter of processed water) consist of 1.0 g barium chloride and 10 ml concentrated sulfuric acid.

Allicin and eugenol were obtained from Wako Pure Chemical Industries, Ltd., Japan, while thyme and cinnamon were obtained from Chemical industries in Egypt. All herbal oils were prepared in a final concentration of 10% solution in Dimethyl sulfoxide (DMSO) as a solvent. All prepared herbal oils were sterilized by filtration using a Millipore cellulose filter membrane (0.45Mli pore diameter).

**RESULTS AND DISCUSSION**

**PREVALENCE OF CAMPYLOBACTER IN THE EXAMINED SAMPLES**

In the current study, a total of 190 rectal swabs from cattle (n=85) and sheep (n=105) and 200 samples from chickens (70 intestinal content and 130 cloacal swabs) were bacteriologically examined. The bacteriological examination of the tested samples revealed that out of 85 rectal samples obtained from cattle, 37 (43.52%) were positive. As shown in Table 2, out of 105 samples from sheep, 37 (35.24%) were positive. The results agreed with a prevalence rate reported by (Fernández et al., 2007) in Chile (39.3%) in case of samples obtained from cattle and (Kassa et al., 2006) 38% in case of sheep, near from some
Table 1: Oligonucleotide primers used for amplification of Campylobacter spp. virulence genes with annealing temperatures.

| Reference          | Product (bp) | Annealing temperature | Primer sequence | ID | Organism | Gene     |
|--------------------|--------------|-----------------------|-----------------|----|----------|----------|
| Eunju and Lee, 2009 | 462 bp       | 58°C                  | AAT TGA AAA TTG CTC CAA CTA TG | CeuE | C. coli  | ceuE.F   |
|                    | 589 bp       | 55°C                  | TGA TTT TAT TTT TAG CAG CG | CeuE.R| C. jejuni| MapA.F   |
| Datta et al., 2003 | 855 bp       | 53°C                  | GCT TTA TTT GCC ATT TGT TTT ATT A | flaA  | Campylo- | flapA664 |
|                    | 494 bp       | 55°C                  | CCTCGGTGTCCTGTGTATTTACCC | MapA.R|         | flA1494  |
| Linton et al., 2000 | 672 bp       | 46°C                  | TTAAGAGCAAGATATAGAAGGTG | wlaN  | WlaN-DL39|         |
| Bang et al., 2003  | 165 bp       | 42°C                  | GTTAGCATACGGCGAAGTAAATTACCT | cdtA  | GNW      |         |
|                    | 495 bp       |                       | GTGACACGTGCAAAGTGGCTATTGAGGACAT | cdtB  | IVH      |         |
|                    | 555 bp       |                       | GTGTAAGATACGGTTATTTATTTAAGGACGCA | cdtC  | WMI-R    |         |
|                    | 2212 bp      |                       | GCTTTACTGATGTTCTCTAATTATTGTACAAGGTTG | Cdt | LYA-F    |         |

Table 2: Prevalence of Campylobacter spp. in chickens, cattle and sheep samples.

| Samples       | Source animal species | Chickens | Cloacal swabs (No.130) | Total | Cattle | Sheep |
|---------------|-----------------------|----------|------------------------|-------|--------|-------|
| Intestinal contents (No.70) | No* | % | No | % | No | % | No | % |
| Positive      | 40   | 2 | 106 | 53 | 146 | 73 | 37 | 43.52 |
| Negative      | 30   | 15 | 24 | 12 | 54 | 27 | 48 | 56.48 |
| Total         | 70   | 35 | 130 | 65 | 200 | 100 | 85 | 100 |

* No; number of samples, %; the percentage of positive/total tested samples.

records all over the world (Fernández and Hitschfeld, 2009; Premaratne et al., 2017). This increase in the shedding of Campylobacter may refer to stress such as lambing, weaning, changing feed, animal movement or transport and environmental contamination originated from wildfowl excreta contaminating the animal water sources (Sutherland et al., 2009). Conversely, lower prevalence rates were reported by (Sutherland et al., 2009) and (Huber et al., 2015).

In chickens, the results revealed that 146 (73%) out of 200 examined samples were bacteriologically positive (Table 2) and these results are consistent with previous authors reported 76% (Bernadette et al., 2012) and 78% prevalence rates (Reddy and Zishiri, 2018). A higher prevalence of 87.2% (Wieczorek et al., 2012) and 96% was reported (Guessoum et al., 2016), while prevalence as low as was 60.2% (Simango, 2013) and 44.9% (Vaishnavi et al., 2015) were also reported. This variation in results among authors may be attributed to that shedding of Campylobacter spp. is of a seasonal pattern (Kassa et al., 2006). Generally, the high prevalence in this study coincides with the sampling period (through a 4-monthes period, October 2016 through January 2017) and/or to hygienic conditions under which tested animals were kept. This can be better explained considering the report of Chanyalew et al. (2013)
who found that the occurrence of sheep fecal carriage by Campylobacter spp. were more prominent in the period August through December. Also, Englen et al. (2007) reported that the prevalence of Campylobacter in broiler flocks was significantly lower during January and February than that from September to December. Nevertheless, the high fecal carriage rates in the examined livestock and poultry increase their potential for being infective for other susceptible individuals and allowing Campylobacters to invade the human food chain as well.

**Typing and molecular identification of the isolated Campylobacter species**

Analysis of Campylobacter species identified in the present study revealed that the C. coli is more prevalent than C. jejuni (13.5 and 21.6%, respectively) in case of cattle and sheep (Table 3). Similar findings were reported by (Sanad et al., 2011) who isolated C. coli (72.9%) more than C. jejuni (39.2%) from fecal samples of dairy cattle in USA, (Uaboi-Egbenni et al., 2011) who also recovered more C. coli (25.9%) than C. jejuni (3.4%) from fecal samples of non-diarrheic goat in South Africa and (Karikari et al., 2017) in Ghana and Niger. These findings confirm the assumption that C. coli are more common in Africa (LaGier et al., 2004).

| Species     | Source animal species | Tested isolates | Positive no. (%) | C. coli ceuE | C. jejuni mapA |
|-------------|-----------------------|----------------|------------------|--------------|---------------|
| Chicks      |                       | 30             | 20 (66.7%)       | 14           | 6             |
| Sheep       |                       | 6              | 6 (100%)         | 3            | 3             |
| Cattle      |                       | 5              | 4 (80%)          | 1            | 3             |
| Total       |                       | 30             | 20 (66.7%)       | 14           | 6             |

* no; number, %; the percentage of positive/total tested samples.

In this study, randomly selected five C. jejuni and C. coli strains isolated from cattle, sheep, and chickens' fecal samples in Beni-Suef governorate were molecularly characterized. For this purpose, thirty typed Campylobacter species were subjected for typing PCR. As shown in Table 4, 52.6% of the tested Campylobacter species from chicken were confirmed by PCR, while 80% and 100% of sheep and cattle isolates, respectively were also confirmed. Seven virulence genes essential in the pathogenesis of Campylobacter spp. were identified by the PCR methodology. One of the best characterized Campylobacter pathogenic markers is the flaA gene which determines flagella formation, therefore bacteria motility and enterocyte colonization (Nuijten et al., 2000). Our results showed that flaA was detected in (100%) of analyzed strains (Table 5). Several reports obtained the same results (Wieczorek and Osek, 2011) and (Ghorbanalizadgan et al., 2018). All these results may suggest that the flaA gene product is necessary for bacterial colonization of animal alimentary tract and determines the stability of bacteria on the surface of contaminated poultry carcass (Wieczorek and Osek, 2011).

Toxins produced by Campylobacter might be another factor that possibly plays a role in disease development. CDT toxin is composed of three subunits: CdtA, CdtB, and CdtC which are necessary to induce a cytotoxic effect in vitro (Martinez et al., 2006). The three cdt genes subunits were detected in all tested strains (Table 5) in consistence with previous literature (Fernandes et al., 2010; Khoshbakht et al., 2013).

The high prevalence rates of virulence genes detected in tested isolates demonstrate their significant role in the pathogenicity of Campylobacter species (Reddy and Zishiery, 2018). The invasiveness markers of Campylobacter isolates, virB11 gene, localized in the pVir plasmid was not detected in any of the tested isolates possessed this gene. These results resemble those reported by other investigations (Feng et al., 2009; Khoshbakht et al., 2013). However, other studies showed a detection rate of 7-20% in Campylobacter spp. (Bang et al., 2003; Datta et al., 2003; Wieczorek and Osek, 2008).
Table 5: Virulence genes detection by multiplex PCR.

| Sample ID | Source | Tested isolates | flaA | VirB11 | wlaN | Cdt cluster | cdtA | cdtB | cdC | Cdt |
|-----------|--------|----------------|------|--------|------|-------------|------|------|-----|-----|
| 1         | Chickens | C. coli       | +    | -      | -    | +           | +    | +    | +   | +   |
| 2         | Sheep   | C. coli       | +    | -      | -    | +           | +    | +    | +   | +   |
| 3         |         | C. jejuni     | +    | -      | -    | +           | +    | +    | +   | +   |
| 4         |         | C. jejuni     | +    | -      | -    | +           | +    | +    | +   | +   |
| 5         |         | C. coli       | +    | -      | -    | +           | +    | +    | +   | +   |

These differences may be due to the plasmid nature of virB11 and the genetic variations of the isolates from various geographical areas (Carvalho et al., 2001).

The putative virulence wlaN gene is probably involved in the expression of ganglioside mimics in Guillain–Barré syndrome and heptosyltransferase and β-1, 3-galactosyltransferase production (Linton et al., 2000; Datta et al., 2003) and results showed that none of the analyzed isolates carried this gene also.

Among all the investigated herbal oils, eugenol was found as the most effective one against Campylobacter spp, meanwhile, thyme as 1500 µg/ml was able to inhibit the growth of 70% of the tested isolates. The results are in harmony with (Rattanachaikunsopon et al., 2010; Grill et al., 2012) who reported that the MIC of egunol oil when tested on C. jejuni was 0.12%. A comparable result of

Antibacterial Effect of Herbal Oils on Campylobacter spp.

The antimicrobial properties of some natural herbal oils against Campylobacter spp. was investigated. A total of 20 confirmed highly virulent Campylobacter spp. strains (14 C. coli and 6 C. jejuni) were tested to determine the minimum inhibitory concentration (MIC). The results showed that MIC values were 500, 1000, 1500, and 2000 µg/ml for eugenol, cinnamon, allicin, and thyme, respectively. Primarily, the obtained results of EOs against Campylobacter spp. confirms the previous concept that EOs are safe antimicrobial agents against a wide variety of pathogenic bacteria; Staphylococcus aureus, Escherichia coli, Listeria monocytogenes and Pseudomonas aeruginosa (Aminzare et al., 2017), L. monocytogenes (Tajik et al., 2015), Listeria monocytogenes and Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium (Raeisi et al., 2016), and and Clostridium perfringens (Aminzare et al., 2018).

Figure 1: Agarose gel electrophoresis of cdt cluster, cdtA and cdtB PCR Products.
Abbreviation: L: Ladder, Pos: Positive control, Neg: Negative control, lanes 1-5, tested Campylobacter Species samples.

Figure 2: Agarose gel electrophoresis of FlaA, VirB11, and WlaN gene PCR.
Abbreviation: L: Ladder, Pos: Positive control, Neg: Negative control, lanes 1-5, tested Campylobacter Species samples.
Eugenol, the main component of clove EO, is thought by several authors to be responsible for the strong biological and antimicrobial properties of clove EO (Catherine et al., 2012). It has been proven to have effective anti-Campylobacter activity (Thanissery et al., 2014). In the present study, the MIC of cinnamon oil was 1000 µg/mL. Thyme which is main component of carvacol was found to be the least effective used oil as its MIC was 2000 mg/mL at 1500 µg/mL it was able to inhibit the growth of 70% of the tested isolates. A MIC of 0.2% value was previously recorded by (Ravishankar et al., 2008) for carvacol. However, both cinnamon and carvacol are phenolic compounds, with both hydrophobic and hydrophilic properties, interact with the lipid bilayer of the bacterial cytoplasmic membrane causing loss of its integrity (Zhu et al., 2016). More detectable efficacy of cinnamon, as a natural preservative agent in meat products, was previously documented in a combinational effect with grape seed extract (Aminzare et al., 2018). Additionally, even subinhibitory concentrations of carvacol are thought to block effectively the bacterial motility and invasion of eukaryotic cells which are considered key steps in C. jejuni infection, by interfering with flagella function without disturbing intracellular ATP levels. This concept broadens the spectrum of antimicrobial activity of carvacol and supports the potential of the compound for use in novel infection prevention strategies (Van Alphen et al., 2012).

The current study showed also that allicin, the main component of garlic, induced its activity against bacteria at a MIC 1500 µg/mL. These results are well in line with those of (Rattanachaikunsopon et al., 2010) who studied the MIC of garlic and cinnamon oils on Campylobacter spp. and declared a MIC of 0.25 to 1 and 0.42 to 1 % respectively for the two oils. From the present study, it is possible to confirm the previous observations of (Kollanoor-Johny et al., 2010) who found that thymol, eugenol and carvacol were all independently effective in significantly reducing Campylobacter concentrations. They remarked that Campylobacter reduction occurred after 15s of incubation in vitro, 20 mM (approximately 0.3%). After 8hr incubation, 10 mM concentrations of cinnamaldehyde, thymol, eugenol, or carvacol were enough in decreasing C. jejuni by at least 5-log colony forming units (CFUs)/mL.

It is important to consider that eugenol, cinnamon, allicin, and thyme have a valuable activity against Quorum sensing (QS). In addition, essential oils might have a role as efflux pump inhibitors. QS is one mechanism in Campylobacter that is associated with biofilm formation. As QS communication has been linked to bacterial proliferation in foods and food spoilage, QS inhibition is a promising target to control Campylobacter and to ensure food safety (Nazzaro et al., 2013; Duarte et al., 2016). Essential oils have new anti-pathogenic drugs principle because of their anti-QS activity might be important in reducing virulence and pathogenicity of drug resistant bacteria in vivo. The mechanisms of action of the EOs include the degradation of the cell wall, damaging the cytoplasmic membrane, cytoplasm coagulation, damaging the membrane proteins, increased permeability leading to leakage of the cell contents, reducing the proton motive force, reducing the intracellular ATP pool via decreased ATP synthesis and augmented hydrolysis that is separate from the increased membrane permeability and reducing the membrane potential via increased membrane permeability (Nazzaro et al., 2013). On the other hand, some phenolic compounds present in several herbal oils act as efflux pump inhibitor, significantly reduce the expression of the CmeABC efflux pump and, therefore, could be used synergistically with antibiotics to inhibit Campylobacter spp. by impacting both antimicrobial influx and efflux (Oh and Jeon, 2015).

CONCLUSION

This study highlights the high prevalence rates of fecal carriage of Campylobacter spp. detected in cattle, sheep and poultry, which indicate the wide-spread of infection in Beni-Suef Governorate, Egypt. The potential role of these animals as significant reservoirs of infection to humans need to be further investigated. The molecular characterization of randomly selected C. jejuni and C. coli local strains revealed that C. coli were more predominant than C. jejuni. The high detection rates of virulence genes probably indicate their significant role in the pathogenicity of the isolated Campylobacter strains. Finally, the studied essential oils were found effective against the virulent Campylobacter strains at MIC of 500, 1000, 1500, and 2000 µg/mL for eugenol, cinnamon, allicin, and thyme, respectively. These
data emphasizes the significance of these oils as natural antimicrobial agents.

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AUTHORS CONTRIBUTION

All authors contributed equally in the planning of the study, drafting the manuscript. All of them approve the final version of the article.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

• Aminzare M, Aliakbarlu J, Tajik H (2015). The effect of Cinnamomum zeylanicum essential oil on chemical characteristics of Lyoner-type sausage during refrigerated storage. Vet. Res. Forum. 6: 31–39. PMC4405683

• Aminzare M, Hashemi M, Hassanazad A, Amir E, Abbasi Z (2017). Antibacterial activity of corn stalk films incorporated with Zataria multiflora and Bonium persicum essential oils. Ann. Res. Rev. Biol. 19: 1–9. https://doi.org/10.9734/ARRB/2017/37103

• Aminzare M, Tajik H, Aliakbarlu J, Hashemi M, Raeisi M. (2018). Effect of cinnamon essential oil and grape seed extract as functional–natural additives in the production of cooked sausage-impact on microbiological, physicochemical, lipid oxidation and sensory aspects, and fate of inoculated Clostridium perfringens. J. food safe. 38: e12459. https://doi.org/10.1111/jfs.12459

• Ampalang RK, Boreux R, Melin P, Akir NI, Bitiang K, Daube GA, De Mol P (2014). Prevalence of Campylobacter among goats and retail goat meat in Congo. J. Infect. Dev. Countries. 8(2):168–175. https://doi.org/10.3855/jidc.3199

• Bang DD, Nielsen EM, Scheutz F, Pedersen K, Handberg K, Madsen M (2003). PCR detection of seven virulence and toxin genes of Campylobacter jejuni and Campylobacter coli isolates from Danish pigs and cattle and campylobacter dissolving toxin production of the isolates. J. Appl. Microbiol., 94:1003–1014. https://doi.org/10.1046/j.1365-2672.2003.01926.x

• Bernadette GG, Essoh, AE, Solange KNGE., Natalie G, Souleymane B, Sébastien NL, Mireille D (2012). Prevalence and Antimicrobial Resistance of Thermophilic Campylobacter Isolated from Chicken in Côte d’Ivoire. Int. J. Microbiol. 2012, 5. 10.1155/2012/150612. https://doi.org/10.1155/2012/150612

• Carvalho ACT, Ruiz-Palacios GM, Ramos-Cervantes P, Cervantes LE, Jiang X, Pickering LK (2001). Molecular characterization of invasive and noninvasive Campylobacter jejuni and Campylobacter coli isolates. J. Clin. Microbiol., 39: 1353–1359. https://doi.org/10.1128/JCM.39.4.1353-1359.2001

• Christofilogiannisp, 2001. Current Inoculation Methods in MIC Determination. Aquacultu, 196: 297–302. https://doi.org/10.1016/S0044-8486(01)00542-7

• Catherine AA, Deepika H, Negi PS. (2012). Antibacterial activity of eugenol and peppermint oil in model food systems. J. Essent. Oil Res, 24: 481–486. https://doi.org/10.1080/10412905.2012.703513

• Chanyalew Y, Asrat D, Amavisit P, Wiri Loongyai W (2013). Prevalence and antimicrobial susceptibility of thermophilic Campylobacter isolated from sheep at Debre Birhan, North-Shoa, Ethiopia, Kasetarts J. Nat. Sci. 47: 551 – 560.

• Datta S, Niwa H, Itoh K (2003). Prevalence of 11 pathogenic genes of Campylobacter jejuni by PCR in strains isolated from humans, poultry meat and broiler and bovine feces. J. Med. Microbiol. 52: 345–348. https://doi.org/10.1099/jmm.0.05056-0

• Duarte A, Luís A, Oleastro M, Domingues FC (2016). Antioxidant properties of coriander essential oil and linalool and their potential to control Campylobacter spp. Food Control, 61: 115–122. https://doi.org/10.1016/j. foodcont.2015.09.033

• Enjun S, Lee Y (2009), Comparison of Three Different Methods for Campylobacter Isolation from Porcine Intestines. J. Microbiol. Biotechnol., 19: 647–650.

• Fernández H, Vera F, Villanueva MP (2007). Arcobacter and Campylobacter species in birds and mammals from Southern Chile. Arch. Med. Vet., 39: 163–165. https://doi.org/10.1128/JCM.39.4.1353-1359.2001

• Feng X, Yuan J, Fei X, Xiao-Rong Z, Jun L, Chang-Qing Z, Hao C (2009). Isolation and characterization of Campylobacter from red-crowned cranes in China. J. Anim. Vet. Adv, 12: 2442-2446.

• Fernández H, Hitchensfield M (2009), occurrence of Campylobacter jejuni and Campylobacter coli and their biotypes in beef and dairy cattle from the south of Chile. Braz. J. Microbiol. 40: 450–454. https://doi.org/10.1590/S1517-83822009000300005

• Fernandes M, Men A, Silva J, Teixeira P (2010). Study of cytolethal distending toxin (cdt) in Campylobacter coli using a multiplex polymerase chain reaction assay and its distribution among clinical and food strains. Foodborne Pathog Dis. 7(1):103-106. https://doi.org/10.1089/fpd.2009.0326

• Grilli E, Vitari F, Domeneghini C, Palmonari A, Tosi G, Guessoum M, Guechi Z Aigoun F, Mahrane S, Hachemi G, De Mol P (2014). Prevalence and Antimicrobial Resistance of Thermophilic Campylobacter jejuni and Campylobacter coli isolates from Danish pigs and cattle and cytolethal distending toxin (cdt) in Campylobacter coli using a multiplex polymerase chain reaction assay and its distribution among clinical and food strains. Foodborne Pathog Dis. 7(1):103-106. https://doi.org/10.1089/fpd.2009.0326

• J. Appl. Microbiol. 114:308-317. https://doi.org/10.1111/jam.12053

• Guessoum M, Guex Z, Aigoun F, Mahrane S, Hachemi A (2016). Campylobacter in sheep, calves and broiler chickens in the central region of Algeria: Phenotypic and antimicrobial resistance profiles. African J. Microbiol. Res. 10:1662–1667. https://doi.org/10.5897/AJMR2016.8238

• Gorbanalizadgan M, Bakhshi B, Najar-Peerayeh S (2018). Heterogeneity of cytolethal distending toxin sequence
types of Campylobacter jejuni and correlation to invasion/cytotoxicity potential: The first molecular survey from Iran. JIVR 15:138-144.

- Harana M, Sasaki Y, Murakami M, Ikeda A, Kusukawa M, Ito K, Asai T, Yamada Y (2011). Prevalence and antimicrobial susceptibility of Campylobacter in broiler flocks in Japan. Zoonoses Public Health. 58(4):241-245. https://doi.org/10.1111/j.1600-0775.2011.01441

- Hemaiaiwa SH, Doble M (2009). Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. Int. J. Phytother. Phytopharmacol. 16(11):997-1005. https://doi.org/10.1016/j.ijpymed.2009.04.006

- Huber R, Gregory L, Beraldi F, Carvalho AFD, Pinheiro NM, van Putten JPM (2012). The Natural Antimicrobial Carvacrol Inhibits Campylobacter jejuni Motility and Infection of Epithelial Cells. PLoS ONE 7(9): e45343. https://doi.org/10.1371/journal.pone.0045343

- Marks M (2014). Raw garlic acts like a natural antibiotic. in Food News

- Martínez I, Mateo E, Churruca E, Girbau C, Alonso RM, Fernández-Astorga, A. 2006. Detection of cdTA, cdTB, and cdTC genes in Campylobacter jejuni by multiplex PCR. Int. J of Medical Microbiology. 296 (1): pp. 45-48. https://doi.org/10.1016/j.mcm.2005.08.003

- Mackiew E, Korsak D, Rzewuska K, Tomczuk K, Rozynek E. 2012. Antibiotic resistance in Campylobacter jejuni and Campylobacter coli isolated from food in Poland. Food Control 23: 297–301. https://doi.org/10.1016/j.foodcont.2011.08.022

- Bazarzad M, Barzegar M, Satarti M. 2013. Cinnamomum zeylanicum essential oil as a natural antioxidant and antibacterial in cooked sausage. J. Food Biochem., 37(1): 62-69. https://doi.org/10.1111/j.1745-4511.2014.00600.x

- Munier M, Guyard-Nicodème M, Hirchaud E, Parra A, Chemaly M, Dory D. 2016. Identification of novel vaccine candidates against Campylobacter through reverse vaccinology. J. Immunol. Res. 5: 715-790. https://doi.org/10.1155/2016/5715790

- Naggić T, Hajsig D, Madić J, Pinter I. 2005. Veterinary Microbiology: Faculty of Veterinary Medicine, University of Zagreb, Croatian Microbiol. Soc. Zagreb.

- Nazzaro F, Fratinni F, De Martino L, Coppola R, De Feo V, Martínez I, Mateo E, Churruca E, Girbau C, Alonso RM, Fernández-Astorga, A. 2006. Detection of cdTA, cdTB, and cdTC genes in Campylobacter jejuni by multiplex PCR. Int. J of Medical Microbiology. 296 (1): pp. 45-48. https://doi.org/10.1016/j.mcm.2005.08.003

- Oh E, Jeon B (2015). Synergic anti-Campylobacter jejuni activity of fluorquinolone and macrolide antibiotics with phenolic compounds. Front. Microbiol. 6: 11-29. https://doi.org/10.3389/fmicb.2015.01129

- Premarathne JMK, Anuar AS, Thung TY, Satharasinghe DA, Jambari NN, Abdul-Mutalib NA, Hua Q, Basri DF, Rukayadi Y, Nakaguchi Y, Nishibuchi M, Radu S (2017). Prevalence and antibiotic resistance against tetracycline in Campylobacter jejuni and C. coli in cattle and beef meat from Selangor, Malaysia. Front. Microbiol., 8: 22-54. https://doi.org/10.3389/fmicb.2017.02254

- Ravishankar S, Zhu L, Law B, Joens L, Friedman M (2008). Plant-derived compounds inactivate antibiotic-resistant Campylobacter jejuni strains. J. Food Protect. 71:1145-1149. https://doi.org/10.4315/0362-028X-J-71-6.1145

- Rivera-Amill V, Kim BJ, Seshu J, Konkel ME (2001). Secretion of the virulence-associated Campylobacter invasion antigens and Campylobacter jejuni requires a stimulatory signal. J. Infect. Dis. 183: 1607-1616. https://doi.org/10.1086/320704

- Rosenquist H, Nielsen NL, Sommer HM, Nørrung B, Christensen BB (2003). Occurrence of virulence genes and strain diversity of Campylobacter jejuni strains. J. Food Prot. 66: 1451-1474. https://doi.org/10.4315/0362-028X-J-66-6.1451
3

• Raci, Tajik, Aminzare M, Sangin Abadi S, Yarahmadi A, Yarahmadi E, Tepe B (2016). The role of nisin, monolaurin, and EDTA in antibacterial effect of Rosmarinus officinalis L. and Cinnamomum zeylanicum blume essential oils on foodborne pathogens. Journal of Essential Oil Bearing Plants. 19(7):1709-20. https://doi.org/10.1080/0972060X

• Rattanachaikunsopon P, Plumkhachorn P (2010). Potential of coriander (Coriandrum sativum) oil as a natural antimicrobial compound in controlling Campylobacter jejuni in raw meat. BioSci. Biotechnol. Biochem., 74: 31–35. https://doi.org/10.1271/bbb.90409

• Reddy S, Zishiri OT (2018). Genetic characterization of virulence genes associated with adherence, invasion, and cytotoxicity in Campylobacter spp. isolated from commercial chickens and human clinical cases. Onderstepoort J. Vet. Res., 15: e1-e9. https://doi.org/10.4102/ojvr.v15i1.1507

• Smith-Palmer AJ, Stewart, Fyfe L. (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett. Appl. Microbiol. 26:118–122. https://doi.org/10.1046/j.1472-765X.1998.00303.x

• Snelling WJ, Matsuda M, Moore JE, Dooley JS (2005). Campylobacter jejuni. Lett. Appl. Microbiol. 37: 297-302. https://doi.org/10.1111/j.1472-765X.2005.01788.x

• Sutherland SJ, Gray JT, Menzies PI, Hook SE, Millman ST. (2009). Transmission of foodborne zoonotic pathogens to riparian areas by grazing sheep. Can. J. Vet. Res. 73:125-31. Publ. Med. PMID: 19436581; PubMed Central PMCID: PMC2666317.

• Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011). Foodborne illness acquired in the United States--major pathogens. Emerg. Infect. Dis. 17(1): 7-15. https://doi.org/10.3201/eid1701.09-1101p1

• Sanad YM, Kassem II, Abley M, Gebreyes W, Lejeune JT, Rajashekar G (2011). Genotypic and phenotypic properties of cattle-associated Campylobacter and their implications to public health in the USA. PLoS One. 6 (10): e25778. https://doi.org/10.1371/journal.pone.0025778

• Sahin O, Fitzgerald C, Stroika S, Zhao S, Sippy RJ, Kwan P, Plummer PJ, Han J, Yaeger MJ, Zhang Q (2012). Molecular evidence for zoonotic transmission of an emergent, highly pathogenic Campylobacter jejuni clone in the United States. J. Clin. Microbiol. 50(3):680–687. https://doi.org/10.1128/JCM.06167-11

• Simango C (2013). Antimicrobial susceptibility of Campylobacter species South. Afr. J. Epidemiol. Infect. 2: 139–142. https://doi.org/10.1080/10158782.2013.1144153

• Thanissery R, Smith DP (2014). Marinade with thyme and orange oils reduces Salmonella Enteritidis and Campylobacter coli on inoculated broiler breast fillets and whole wings. Poult. Sci. 93:1258–1262. https://doi.org/10.3382/ps.2013-03697

• Tajik H, Aminzare M, Mounesi Raad T, Hashemi M, Hassanzad Azar H, Raesi M, Naghili H (2015). Effect of Zataria multiflora Boiss Essential Oil and Grape Seed Extract on the Shelf Life of Raw Buffalo Patty and Fate of Inoculated Listeria monocytogenes. J. Food Proc. Preserv. 39: 3005–3013. https://doi.org/10.1111/jfpp.12553

• Uaboi-Eghenni PO, Besong A, Samie, Obi CL. (2011). Prevalence and antimicrobial susceptibility profiles of Campylobacter jejuni and c. coli isolated from diarrheic and nondiarrheic goat feces in Venda region, South Africa, Afr. J. Biotechnol. 10: 14116–14214. https://doi.org/10.5897/AJB10.1662

• Vaishnavi C, Singh M, Kapoor P (2015). Isolation of Campylobacters from Intestinal Tract of Poultry in Northern Region of India. Adv. Microbiol. 5: 797–806. https://doi.org/10.4236/am.2015.512084

• Wietczorek K, Osek J (2008). Identification of virulence genes in Campylobacter jejuni and C. coli isolates by PCR. Bull. Vet. Inst. 52: 211–216.

• Wietczorek K, Osek J, Brno AV (2011). Molecular characterization of Campylobacter spp. isolated from poultry feces and carcasses in Poland. Acta Vet. Brno. 80: 019–027. https://doi.org/10.2754/avb2011080010019

• Wietczorek K, Szewczyk R, Osek J (2012). Prevalence, antimicrobial resistance, and molecular characterization of Campylobacter jejuni and C. coli isolated from retail raw meat in Poland. Vet. Med. 57: 293. https://doi.org/10.17221/6016-VETMED

• Xiaonan Lu, Derrick R, Samuelson, Barbara A, Rasco, Michael EK (2012). Antimicrobial effect of diallyl sulphide on Campylobacter jejuni biofilms. J. Antimicrob. Chemotherpy. 67(8): 1915–1926. https://doi.org/10.1093/jac/dks138

• Zhu H, Du M, Fox I, Zhu MJ (2016). Bactericidal effects of cinnamon cassia oil against bovine mastitis bacterial pathogens. Food Control. 66: 291–299. https://doi.org/10.1016/j.foodcont.2016.02.013