**Implication of Turkey Broiler Flocks in Prevalence of Antibiotic Resistance *Capmpylobacter Spp*.**

Samah Eid1, Nayera M. Al-Atfeehy1, Abdel Hafeez Samir1 and Hefny Y. Hefny2

1Reference Laboratory for Veterinary Quality Control on Poultry Production- Animal Health Research Institute, Egypt

2Animal Health Research Institute- Zagazig Provincial Laboratory, Egypt

samaheid@ymail.com

**Abstract:** A total of 100 samples were collected from diseased fattening turkeys, samples included fecal swabs, liver, and intestine were subjected to conventional examination for *campylobacter species* identification, isolates were confirmed by PCR through the detection of *cad* F gene the conserved for genus *campylobacter*, *ceu* Egene specific for *campylobacter coli*, and *C*j gene specific for *campylobacter jejuni,* the results revealed that **16/100 (16%)** of samples were positive for *campylobacter species***, 9/16 (56.2%), 7/16(43.8%)** of isolates belong to *campylobacter jejuni, and campylobacter coli,* respectively. Phenotypic and genotypic antibiotic resistance attributes of isolates were studied by disc diffusion and PCR. The results revealed that **16/16 (100%)** of isolates showed antibiotic resistance patterns to ampicillin, tetracycline, and erythromycin. Resistance rates against cefotaxime and gentamycin were **(81.3%), (87.5, %),** respectively. Only **3/16 (18.8%)** of isolates showed resistance rate against imipenem, **16/16 (100%)** isolates demonstrated profiles of multidrug resistant strains. Studying the genetic antibiotic resistance attributes of isolates by PCR revealed that **10/16 (62.5%), 9/16 (56.2%)** of isolates have ***tet*O** gene for tetracycline resistance, and ***cme*B** gene for efflux pump, respectively. PCR failed to detect ***bla*OXA** gene for betalactams. The findings raised concerns due to the presence of circulating *campylobacter spp* in turkey farms that may impose a potential high public health risk caused by their zoonotic nature, furthermore disseminate antibiotic resistance genes against key antibiotics used in veterinary and human medicine.

[Samah Eid, Nayera M. Al-Atfeehy, Abdel Hafeez Samirand Hefny Y. Hefny. **Implication of Turkey Broiler Flocks in Prevalence of Antibiotic Resistance *Capmpylobacter Spp*.** *Researcher* 2018;10(3):60-67]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). <http://www.sciencepub.net/researcher>. 8. doi:[10.7537/marsrsj100318.08](http://www.dx.doi.org/10.7537/marsrsj100318.08).

**Key words**:antimicrobial resistance, PCR, *capmpylobacter spp*, turkeys

**1. Introduction**

*Campylobacter spp* are motile spirally curved, Gram negative bacteria that are commonly present in the intestinal tract of domestic and wild animals **(Blaser** and **Engberg, 2008)** *Campylobacter jejuni* and, *Campylobacter coli* are the most important pathogenic species, they grow in a micro-aerophilic atmosphere with 10% CO2 and 5% O2, at a narrow temperature range between 30∘C - 46∘C, and thus classified as thermophilic campylobacters **(Allos, 2001).**

Campylobacter is part of the normal flora living in the intestines of healthy chickens and other animals. During slaughtering and gutting chickens, the contents of intestines, including the Campylobacter, could contaminate raw chicken meat**.**

Many studies confirmed the risk of contamination of poultry carcass, meat and meat products at the time of slaughter and processing, in this regard, **(Alexandra, 2009)** concluded that *Campylobacter* is present in the crop at 104 and in the ceca at 107 CFU/g contents; while the estimated *Campylobacter* infectious dose for humans is 500 cells. **Viktoria *et al.* (2007)** studied the prevalence of *Campylobacter* in samples collected from turkey carcasses at slaughter house they found that over one-quarter (29.2%) of the tested samples were Campylobacter positive**.**

*Campylobacter* can be easily spread from bird to bird through a common water source or through contact with infected feces. *Campylobacter* can also be present in the giblets, especially the liver **(CDC, 2015).**

*Campylobacter* bacteria are a major cause of foodborne diarrheal illness in humans and were the most common bacteria that cause gastroenteritis worldwide, in developed and developing countries. The high incidence, the disease course duration and the sequelae, makes campylobacteriosis highly important from a socio-economic perspective **(WHO, 2015).**

Campylobacterios is most reported symptoms are diarrhea, cramping, abdominal pain, and fever within two to five days after exposure, bloody diarrhea accompanied by nausea and vomiting, the disease course lasts for about one week **(CDC, 2015).** In developing countries, infections are commonly detected in children younger than two years old, sometimes resulting in death, *Campylobacter* species are prevalent in food animals such as poultry. The main route of transmission is believed to be foodborne via undercooked meat and meat products, often carcasses or meat are contaminated from feces during slaughtering **(WHO, 2015).**

Campylobacteriosis is estimated to affect over 1.3 million persons every year mainly in summer, although Campylobacter infection does not commonly cause death, but it has been estimated that approximately 76 persons with Campylobacter infections die each year **(CDC, 2015). Nachamkin, (1998**) concluded that *Campylobacter jejuni* not only is an important cause of bacterial gastroenteritis in humans but also has been associated with Guillain-Barré syndrome, which is an acute immune-mediated demyelinating disorder of the peripheral nervous system.

The occurrence of high resistance to several antimicrobials, especially key drugs for the treatment of human campylobacteriosis, representing a potential risk for public health, also the emergence of antimicrobial resistance among *Campylobacter* isolates recovered from turkeys has increased dramatically, thus becomes a growing public health issue **(El-Adawy *et al.,*2012).**

Poultry is widely recognized as a major reservoir in cases of Campylobacteriosis, due to symptomless carriage in the live bird. The problem is exacerbated by intensive rearing. Moreover, usage of antimicrobials in poultry production, for prophylactic, therapeutic or performance-enhancing purposes, contributes to the development of resistance in pathogens, which can have serious consequences for the treatment of human illness.

This study was aimed to investigate the prevalence of *Campylobacter* *spp* in turkeys and to assess the phenotypic and genotypic antimicrobial resistance (AMR) attributes of isolates.

**2. Materials and Methods**

**2.1 Sampling**

A total of 100 samples were collected from diseased turkeys with history of digestive symptoms (60 coloacal swabs, 20 liver, and 20 intestines) from Belbeis, Sharqia governorate, Egypt in Summer 2017.

**2.2 Isolation and Identification *Campylobacter species***

Isolation and identification of *Campylobacter spp* were applied according to (**ISO 10272-1 2006)**.

# 2.3 PCR technique for confirmation of genus Campylobacter and, Campylobacter species identification

# 2.3.1. Extraction of DNA: QIAamp DNA Mini Kit, catalogue no.51304 was used.

**2.3.2 PCR Master Mix:** Emerald Amp GT PCR master mix (Takara) code no. RR310A.

**2.3.3. Oligonucleotide primers:** Metabion (Germany) with specific sequence for tested genes were used, primer sequences and thermal cycling condition as demonstrated in table (1).

**2.4. Antibiogram of campylobacter isolates:** All campylobacter isolates were tested for their susceptibility against 7 antibiotic agents’ **ampicillin, imipenem, cefotaxime, cefoxitin**, **erythromycin**, g**entamycin**, and t**etracycline (Oxoid),** by disc diffusion method according to **(Quinn *et al.,* 1999)**.

**2.5. PCR investigation of antibiotic resistance genotypic attributes**: by using Oligonucleotide primers, Metabion (Germany), primer sequences and thermal cycling condition as demonstrated in table (1).

**Table 1. Oligonucleotide sequences and thermal profiles used in PCR**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test target**  | **Tested gene** | **Primer sequence (5´-3´)** | **Amplicon size** | **Thermal profile** | **Reference** |
| ***Genus campylobacter*** | ***CadF*** | **F: TGGAGGGTAATTTAGATATG****R: CTAATACCTAAAGTTGAAAC** | **400 bp** | **94ͦ C, 5 min; 35 cycles: 94ͦ C, 1 min; 45ͦ C, 1 min;** **72ͦ C, 3 min; And, 72ͦ C, 10 min** | ***Konkel et al.* (1999)** |
| ***Campylobacter coli*** | ***CeuE*** | **F: ATGAAAAAATATTTAGTTTTTGCA****R: ATTTTATTATTTGTAGCAGCG** | **894 bp** | **94ͦ C, 5min, 35 cycles:94ͦ C, 1 min; 57ͦ C, 1 min;** **72ͦ C, 1 min; And, 72ͦ C, 10 min** | **Gonzalez *et al.* (1997)** |
| ***Campylobacter jejuni*** | ***CJ*** | **F:-GAGTAAGCTTGGTAAGATTAAAG****R: AAGAAGTTTTAGAGTTTCTCC** | **500 bp** | **94ͦ C, 5min, 35 cycles:94ͦ C, 1 min; 53ͦ C, 1 min;****72ͦ C, 1 min; And, 72ͦ C, 10 min** | **Rantsioua *et al.* (2010)** |
| ***Tetracycline resistance***  | ***tet O*** | **F: AACTTAGGCATTCTGGCTCAC****R: TCCCACTGTTCCATATCGTCA** | **515 bp,** | **94ͦ C, 5miN, 35 cycles:94ͦ C, 1 min;56ͦ C, 1 min;****72ͦ C, 1 min; And 72ͦ C, 10 min** | **Abdi-Hachesoo *et al.* (2014)** |
| ***Efflux pump***  | ***cme* B** | **F: 5'-CCTACCTCCTATACCTGG-3'****R: 5'-TTGAACTTGTGCCGCTGG-3'** | **515 bp** | **94ͦ C, 5min,,35 cycles:94ͦ C, 1 min;56ͦ C, 1 min;****72ͦ C, 1 min; And,72ͦ C, 10 min** | **Pamela *et al.* (2006)** |
| ***βlactam resistance***  | ***βla OXA*** | **F-TCGATGGATTGCTTTAATGG****R- TTGTCAAGCCAAAAAGTATCG** | **564 bp** | **94ͦ C, 5min; 35 cycles: 94ͦ C, 1 min; 56ͦ C, 1 min;****72ͦ C, 1 min; And 72ͦ C, 10 min**  | **Alfredson *et al.* (2005)** |

**3. Results**

**Table 2. Prevalence rate of *Campylobacter spp* among examined samples**

|  |  |
| --- | --- |
| **Sample** | **Positive Isolates** |
| **Type** | **Number** | **Number** | **Prevalence** |
| **Fecal swabs** | **60** | **11** | **18.3%** |
| **Liver** | **20** | **2** | **10%** |
| **Intestine** | **20** | **3** | **15%** |
| **Total** | **100** | **16** | **16%** |

**Table 3. Confirmation and Species Identification of *Campylobacter* Isolates by Conventional PCR**

|  |  |  |
| --- | --- | --- |
| **Target test** | **Tested genes** | ***Campylobacter* isolates** |
| **Number** |  **Detection Rate**  |
| ***Campylobacter spp*** | ***Cad* F** | **16** | **100%** |
| ***Campylobacter coli*** | ***Ceu*E** | **7** | **43.8%** |
| ***Campylobacter jejuni*** | ***C*j** |  **9** | **56.2%** |

**Table 4. Phenotypic antibiotic resistance profiles of Campylobacter isolates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibiotic Group** | **Antibiotic Agent** | **Abbrev.** | **Conc.** | **Resistant**  | **Susceptible** |
| **NO** | **%** | **NO** | **%** |
| **β-lactamins** | **Penicillins** | **Ampicillin** | **AM** | **10 µg** | **16** | **100%** | **0** | **0%** |
| **Imipenem** | **IPM** | **10µg** | **3** | **18.8%** | **13** | **81.3%** |
|  | **Cephalosporins** | **Cefotaxime** | **CTX** | **30 µg** | **13** | **81.3%** | **3** | **18.8%** |
|  |  | **Cefoxitin** | **FOX** | **30 µg** | **10** | **62.5%** | **6** | **37.5%** |
| **Macrolydes** |  | **Erythromycin** | **E** | **15 µg** | **16** | **100%** | **0** | **0%** |
| **Aminoglycosides** |  | **Gentamycin** | **CN** | **10 µg** | **14** | **87.5%** | **2** | **12.5%** |
| **Tetracyclines** |  | **Tetracycline** | **TE** | **30 µg** | **16** | **100%** | **0** | **0%** |

**Table 4-a. Phenotypic antibiotic resistance profiles of *Campylobacter coli* isolates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibiotic Group** | **Antibiotic Agent** | **Abbrev.** | **Conc.** | **Resistant**  | **Susceptible** |
| **NO** | **%** | **NO** | **%** |
| **β- lactamins** | **Penicillins** | **Ampicillin** | **AM** | **10 µg** | **7/7** | **100%** | **0** | **0%** |
| **Imipenem** | **IPM** | **10µg** | **0** | **0%** | **7/7** | **100%** |
|  | **Cephalosporins** | **Cefotaxime** | **CTX** | **30 µg** | **6/7** | **85.7%** | **1/7** | **14.3%** |
|  |  | **Cefoxitin** | **FOX** | **30 µg** | **5/7** | **71.4%** | **2/7** | **28.6%** |
| **Macrolydes** |  | **Erythromycin** | **E** | **15 µg** | **7/7** | **100%** | **0** | **0%** |
| **Aminoglycosides** |  | **Gentamycin** | **CN** | **10 µg** | **6/7** | **85.7%** | **1/7** | **14.3%** |
| **Tetracyclines** |  | **Tetracycline** | **TE** | **30 µg** | **7/7** | **100%** | **0** | **0%** |

**Table 4-b. Phenotypic antibiotic resistance profiles of *Campylobacter jejuni* isolates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibiotic Group** | **Antibiotic Agent** | **Abbrev.** | **Conc.** | **Resistant**  | **Susceptible** |
| **NO** | **%** | **NO** | **%** |
| **β-lactamins** | **Penicillins** | **Ampicillin** | **AM** | **10 µg** | **9/9** | **100%** | **0** | **0%** |
| **Imipenem** | **IPM** | **10µg** | **3/9** | **33.3%** | **6/9** | **66.7%** |
|  | **Cephalosporins** | **Cefotaxime** | **CTX** | **30 µg** | **7/9** | **77.8%** | **2/9** | **22.2%** |
|  |  | **Cefoxitin** | **FOX** | **30 µg** | **5/9** | **55. 6%** | **4/9** | **44.4%** |
| **Macrolydes** |  | **Erythromycin** | **E** | **15 µg** | **9/9** | **100%** | **0** | **0%** |
| **Aminoglycosides** |  | **Gentamycin** | **CN** | **10 µg** | **8/9** | **88.9%** | **1/9** | **11.1%** |
| **Tetracyclines** |  | **Tetracycline** | **TE** | **30 µg** | **9/9** | **100%** | **0** | **0%** |

**Table 5. Investigation of the presence of antibiotic resistance genes in isolated *campylobacter spp* by PCR**

|  |  |  |
| --- | --- | --- |
| **Antibiotic group** | **Tested genes** | ***Campylobacter* isolates** |
| ***Campylobacter coli*** | ***Campylobacter jejuni*** | **Total** |
| **Tetracycline** | ***tet*O** | **6/7 (85.7%)** | **4/9(44.4%)** | **10/16(62.5%)** |
| **Efflux pump** | ***cme*B** | **4/7(57.1%)** | **5/9(55.5%)** | **9/16 (56.2%)** |
| **Penicillin** | ***βla* OXA** | **0** | **0** | **--** |

**4. Discussion**

In the present study a total of **100** samples were collected from fattening turkeys at the slaughter age between **150** to **160** day old, samples were examined for *Campylobacter spp* isolation by using conventional bacteriological methods, the results revealed that **16/100 (16%)** of samples were positive for *Campylobacter spp* with a prevalence rate of **(16%).** PCR for the detection of ***cad* F** gene which is a genus specific conserved gene for *campylobacter* was applied in order to confirm the positivity of isolates, PCR targeting ***cad* F** for detection of genus *campylobacter* was also used **by ([Nayak](https://www.ncbi.nlm.nih.gov/pubmed/?term=Nayak%20R%5BAuthor%5D&cauthor=true&cauthor_uid=15797819)*[, et](https://www.ncbi.nlm.nih.gov/pubmed/?term=Nayak%20R%5BAuthor%5D&cauthor=true&cauthor_uid=15797819) al.,* 2005).** In the same regard, almost similar prevalence rate was reported by (**Carmelo *et al.,* 2013)** who detected *Campylobacter spp* from poultry samples with a prevalence rate of **(20.7%)** meanwhile, higher prevalence rate was reported by **(Korsak *et al.,* 2015)** who reported a prevalence rate of **(41.1%)**.

Humans often become infected by zoonotic pathogens as *Campylobacter coli*, and *Campylobacter jejuni* by ingesting contaminated food or water, in this instance raw or uncooked meat, like poultry meat, and contact with animals stand for the main transmission roots (**Blaser** and **Engberg, 2008).** In the current study the identified species were confirmed by using PCR for detection of ***ceu*E**, and ***C*j** genes which are specific for *Campylobacter coli, and Campylobacter jejuni*, respectively. In this instance (**Nayak *et al.,* 2005)** applied PCR for detection of ***ceu*E** gene and the specific undefined gene for species identification of both *Campylobacter coli* and *Campylobacter jejuni* species. PCR results of our investigation demonstrated that **7/16(43.8%)**, and **9/16(56.2%)** of isolates were *Campylobacter coli*, and *Campylobacter jejuni*, respectively. In this instances, nearly similar detection rates of *Campylobacter species* were reported by **(Carmelo *et al.,* 2013**) who reported detection rates of **(48.2 %, and 51.8%)** for *Campylobacter coli*, and *Campylobacter jejuni,* respectively. Variable detection rates of *Campylobacter spp* were recorded by different researchers as **(Engy *et al.,* 2015)** who recorded that **(91.7%)** of the total **36** detected isolates were identified as Campylobacter *coli* and **(8.3%)** *Campylobacter jejuna*. Furthermore, **(Kashoma *et al.,* 2014)** who confirmed that **(72.3%)** of the detected isolates were *campylobacter coli,* **(5.3%)** of isolates were *campylobacter jejuni,* and that **(22.5%)** of isolates as other *Campylobacter spp.*

An emergence of multiple resistance patterns of *Campylobacter species* to several antibiotic classes has been observed globally, the most common antimicrobial agents Macrolides, as erythromycin which is commonly used in the treatment of Campylobacter infections, tetracyclineis considered an alternative choice. However, campylobacter resistance to fluoroquinolones, macrolides, aminoglycosides, and beta-lactams have been developed. (**Hindawi, 2013).** Furthermore, concerns of the demonstrated resistance of *Campylobacter* to the fluoroquinolones that has limited their use as drugs of choice in human medicine and the increasingly detected resistance to macrolides (erythromycin) as an alternative choice, beside the increasingly demonstrated resistance to aminoglycosides, and beta lactamsincluding, penicillin, cephalosporinare increasing in medical, veterinary and scientific domains (**Giacomelli *et al.,* 2014)**.

In the current study, **16/16 (100%)** of isolates showed phenotypic resistance patterns against at least one antimicrobial agent that is classified in three or more antimicrobial group, as **(100%)** of isolates showed resistance against penicillin, erythromycin, and tetracycline, also **(87.5%)**, and **(81.3%)** of isolates showed resistance against gentamycin, and cefotaxime, respectively, consequently the isolates can be considered multidrug resistant strains as defined by ([**EUCAST, 2014**](#_ENREF_6)**)** this result demonstrated the potential high public health risk imposed by these isolates, similar finding was also reported by (**Aarestrup *et al.,* 2011)** who concluded the association of emergent campylobacter resistant strains in human clinical samples with the emergence of antimicrobial resistance observed in animals, the same result was also found by (**Pérez *et al.,*2013)** who described (10.3% ) of their studied isolates as pan-susceptible campylobacter populations, they also reported that multidrug resistance isolates were observed in *Campylobacter coli* compared with *Campylobacter jejuni* **(33.3%** vs. **11.9%),** they also raised their concerns from the public health risk imposed by those populations as they demonstrated resistance against fluoroquinolone, macrolide, and tetracycline.

There was no significant difference in the demonstrated phenotypic resistance profiles observed in this study between the investigated *Campylobacter coli* and *Campylobacter jejuni* isolates, as **(100%)** of isolates from both species demonstrated resistance to penicillin, erythromycin and tetracycline. In the same regards, resistance rates demonstrated to gentamycin were **(88.9%)** and **(85.7%)** for *Campylobacter jejuni* and *Campylobacter coli*, respectively. Also, resistance rates demonstrated against cefotaxime, and cefoxitin were (**77.8%** and**, 55. 6%)** for *Campylobacter jejuni* and **(85.7%,** and **71.4%)** for *Campylobacter coli*. This result, differed from that recorded by (**Kashoma *et al.,* 2014)** who reported that *Campylobacter coli* isolates displayed a higher proportion of resistance than *Campylobacter jejuni* against most antimicrobials.

The results of this study, demonstrated that (**100%**) of isolates from both *Campylobacter coli*, and *Campylobacter jejuni* were resistant to erythromycin, while this result was in agreement with that of **(Engy *et al.,* 2015)** who recorded the prevalence of erythromycin resistance among their isolates and (**Carmelo *et al.,* 2013)** who recorded that (**80.1%)** of their studied Campylobacter isolates demonstrated resistance to erythromycin, the result disagreed with that of (**El-Adawy *et al.,* 2015)** who reported that **(100%)** of *Campylobacter* isolates were susceptible to erythromycin.

**Gibreel *et al.* (2004)** reported that both Kanamycin and tetracycline resistance is mediated by a plasmid that is transferred by conjugation between *Campylobacter strains*. In the current work, there was observed phenotypic resistance to gentamycinin **6/7(85.7%)**, and **8/9(88.9%)** of *Campylobacter coli*, and *Campylobacter jejuni*, respectively. While lower resistance rate was observed by **(Carmelo *et al.,* 2013)** who recorded a resistance rate of **(27.9%)** among the *Campylobacter spp* involved in their study**,** the present result was in contrast to the result reported by (**El-Adawy *et al.,* 2015)** who reported that **(100%)** of the studied *Campylobacter  jejuni*isolates, and *Campylobacter coli* isolates were sensitive to gentamycin.

**Luangtongkum *et al.,* (2006)** reported that, since the use of tetracycline as feed additives in poultry production for both therapeutic and sub therapeutic purposes, it is possible that campylobacter may have evolutionally become resistant to tetracycline, leading to the widespread distribution of tetracycline-resistant campylobacter in animal reservoirs regardless of the production types, theirfinding agreed with the results recorded by this study as **(100%)** of tested *Campylobacter coli*, and *Campylobacter jejuni* isolates demonstrated phenotypic resistance patterns to tetracycline by disc diffusion test, this result agreed with that of (**Giacomelli *et al.,* 2014)** who reported a resistance rate of **(96%)**. Lower resistance rates were observed by **(El-Adawy *et al.,* 2015)** who observed resistance rates of **(44.0%,** and **51.3%)** *Campylobacter coli* and *Campylobacter jejuni,* respectively.

The resistance rate detected for ampicillin were **(100%)** for both **7/*7*** *Campylobacter coli*, and **9/9** *Campylobacter jejuni*, while this result disagreed with that of (**[Ewnetu](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ewnetu%20D%5BAuthor%5D&cauthor=true&cauthor_uid=20482228)** and[**Mihret,**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Mihret%20A%5BAuthor%5D&cauthor=true&cauthor_uid=20482228) **2010)** who detected **a** resistance rate of **(16.6%**) against ampicillin. Almost similar resistance rate was reported by **(Giacomelli *et al.,* 2014)** who recorded the prevalence of ampicillin resistant strains with a rate of **(88%)**.

Resistance rates demonstrated against cefotaxime, cefoxitin, and imipenem were (**77.8%, 55. 6%**, and33.3**%)** for *Campylobacter jejuni* and, were **(85.7%, 71.4%** and, **0%)** for *Campylobacter coli*, respectively. This result agreed with that reported by (**Giacomelli *et al.,* 2014)** who detected resistance rate of **(100%)** for at least three cephalosporin, the result also agreed with that recorded by **(Martin** and **Kaye, 2004)** who found that campylobacter strains can be considered resistant to beta lactams, as penicillin and narrow-spectrum cephalosporin but not to carbapenems.

**Zhang** and **Plummer (2008)** concluded that campylobacter resistance to tetracycline can be attributed to its ability to undergo spontaneous mutations and also its ability to acquire resistance determinants by natural transformation, transduction, or conjugation, as in case of conjugation of 𝑡𝑒𝑡(𝑂)-carrying plasmids. **Connell, (2003)** concluded that resistance of *Campylobacter jejuni* and *Campylobacter coli* to tetracycline is attributed mainly to the acquisition of *tet* (𝑂) gene which encodes ribosomal protection proteins (RPPs). In the present study, PCR technique was applied to investigate the genetic attributes of isolates for tetracycline resistance by detection of ***tet* (o)** gene, the results of PCR was in accordance with those revealed by disc diffusion, in this regard **10/16 (62.5%), 6/7 (85.7%),** and **4/9 (44.4%)** of Campylobacter isolates, *Campylobacter coli*, and *Campylobacter jejuni*, respectively. These results are in agreement with that reported by **(Abdi-Hachesoo *et al.,* 2014)** who recorded detection rates for *tet* (o) gene as followed: **(83.1% 92.5,** and **74.4 %)** for the studied Campylobacter isolates, *Campylobacter coli,* and *Campylobacter jejuni,* respectively. **Engy *et al.* (2015)** also recorded that **9/33 (27.3%)** *Campylobacter coli* isolates were positive for the tetracycline resistance gene *tet (O)*, although only two of these were resistant to tetracycline in the disc diffusion test.

 Macrolides are of the safest and most effective antimicrobial drugs used against most of Gram-positive and the Gram-negative microorganisms, including Campylobacter, their mode of action is to interrupt protein synthesis in bacterial ribosome resulting in inhibition of bacterial RNA-dependent protein synthesis (**Poehlsgaard** and **Douthwaite, 2005).** Conformational changes in the ribosome subsequently, termination of the elongation of the peptide chain is caused by binding of macrolide to the target site in the bacterial 23S rRNA (**Pfister *et al.,* 2004).** The resistance to macrolides can also be mediated by modifications of the ribosomal proteins L4 and L22, resistance to macrolide in *Campylobacter species* is also commonly mediated by efflux pump, in this instance, (**Cagliero *et al.,* 2006)** reported that at least eight efflux systems are identified of which is *cme*ABC multidrug efflux pump that works in synergy with mutations. Furthermore, **(Hindawi, 2013)** mentioned that *cme*ABC multidrug efflux pump are the major efflux mechanism causing macrolides antimicrobial resistance in campylobacters. Resistance rates recorded by disc diffusion for *Campylobacter coli,* and *Campylobacter jejuni* were (**88.9%** and **85.7%),** respectively, this result was in accordance with the result of PCR for detection of ***cme*B** gene which mediates the efflux pump mechanism and mainly mediates macrolide resistance, as **9/16 (56.2%), 4/7(57.1%),** and **5/9(55.5%)** of *Campylobacter isolates, Campylobacter jejuni*, and *Campylobacter coli,* respectively. Furthermore, (**Cagliero *et al.,*2006)** studied the resistance attributes of highly macrolides resistant *Campylobacter strains* with specific target site mutations, they found that inactivation of *cme*ABC resulted in reduced resistance to macrolides in addition, it leads to restored susceptibility to erythromycin, suggesting the significant synergistic function of efflux system with target mutations in acquiring and expression of macrolide resistance in campylobacter.

**Martin** and **Kaye (2004)** confirmed that Beta lactams mode of action is through binding to penicillin binding proteins causing disruption of peptidogly can cross linking in bacterial cell wall leading to cell death. Interestingly, although results of disc diffusion applied in this study revealed that 16/16 **(100%)** of isolates are phenotypically resistant to ampicillin, PCR failed to detect ***βla* OXA** gene, the specific for penicillin resistance in the studied isolates. Studies and researches interpreted the resistance of campylobacter to beta lactams due to multiple mechanisms, in this regards (**Tajada *et al.,* 1996)** attributed beta lactams resistance in *Campylobacter jejuni* and *Campylobacter coli* to their ability to produce beta lactamases, meanwhile (**Lin *et al.,* 2002)** reported that beside the ability to hydrolyze beta lactam ring through production of beta lactamases, resistance in campylobacter strains can be attributed to the action of efflux pumps that is mediated by *cme*ABC genes in the resistant mutants.

**Acknowledgments**

The authors are grateful to Engy A. Bayoumi, the researcher in the Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute for her kind support.

**References**

1. Aarestrup F M, McDermott P F, and Wegener H C. Transmission of antibiotic resistance from food animals to humans, in Campylobacter. I. Nachamkin, C. M. Szymanski, and M. J. Blaser, Eds., pp. 645–665, American Society for Microbiology, Washington, DC, USA, 2008.
2. Abdi-Hachesoo B, Khoshbakht R, Sharifiyazdi H, Tabatabaei M, Hosseinzadeh S, Asasi K. Tetracycline Resistance Genes in *Campylobacter jejuni* and *Campylobacter coli* Isolated From Poultry Carcasses. Jundishapur J Microbiol.: September; 7(9): e12129.
3. Alexandra J. Scupham. Campylobacter Colonization of the Turkey Intestine in the Context of Microbial Community Development. Applied And Environmental Microbiology, June 2009, p. 3564–3571 Vol. 75, No. 11, doi:10.1128/AEM.01409-08.
4. Alfredson DA, Korolik V. Isolation and expression of a novel molecular class D b-lactamase, OXA-61, from *Campylobacter jejuni*. Antimicrob Agents Chemother 2005; 49: 2515 –8.
5. Allos, B. M*. Campylobacter jejuni* infections, update on emerging issues and trends. Clinical Infectious Diseases, vol. 32, no. 8, p p. 1201–1206, 2001.
6. Blaser B. and Engberg J. Clinical aspects of *Campylobacter jejuni* and C*ampylobacter coli* infections, in *Campylobacter*. I. Nachamkin, C. M. Szymanski, and M. J. Blaser, Eds., pp. 99–121, American Society for Microbiology, Washington, DC, USA,2008.
7. Cagliero C, Mouline C, Cloeckaert A, and Payot S. Synergy between efflux pump CmeABC and modifications in ribosomal proteins L4 and L22 in conferring macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrobial Agents and Chemotherapy, vol. 50, no. 11, pp. 3893–3896, 2006.
8. Carmelo G. A. Nobile, Rosa Costantino, Aida Bianco, Claudia Pileggi, Maria Pavia. Prevalence and pattern of antibiotic resistance of *Campylobacter spp.* in poultry meat in Southern Italy. Food Control, Volume 32, Issue 2, August 2013, Pages 715–718. doi:10.1016/j.foodcont.2013.02.011.

# CDC, 2015. *Campylobacter*, Campylobacteriosis.http://www.cdc.gov/nczved/divisions/dfbmd/diseases/ campylobacter/.

1. Connell, S. R. Tracz, D. M. Nierhaus, K. H. and Taylor, D. E. Ribosomal protection proteins and their mechanism of tetracycline resistance. Antimicrobial Agents and Chemotherapy, vol. 47, no. 12, pp. 3675–3681, 2003.
2. El-Adawy H, Ahmed MF, Hotzel H, Tomaso H, Tenhagen BA, Hartung J, Neubauer H, Hafez HM. Antimicrobial susceptibilities of *Campylobacter jejuni* nd *Campylobacter coli* recovered from organic turkey farms in Germany. Poult Sci. 2015 Sep 14. pii: pev259.
3. El-Adawy H, Hotzel H, Düpre S, Tomaso H, Neubauer H, Hafez M. Determination of antimicrobial sensitivities of *Campylobacter jejuni* isolated from commercial turkey farms in Germany. Avian Dis. 2012 Dec; 56(4):685-92.
4. Engy A. Hamed, Mona A. A. Abdel Rahman, Azhar G. Shalaby, Mai M. Morsy, Soad A. Nasef. Antibiotic resistance and polymorphism in the quinolone resistance-determining region of Campylobacter spp. isolated from 1-day-old ducklings. The Veterinary Journal. Available online 28 September 2015. doi:10.1016/j.tvjl.2015.09.020.
5. EUCAST, 2014. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0 http://www.eucast.org.
6. Giacomelli M, Salata C, Martini M, Montesissa C, Piccirillo A. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from poultry in Italy. Microb Drug Resist. 2014 Apr; 20(2):181-8. doi: 10.1089/mdr.2013.0110. Epub 2013 Dec 9.
7. Gibreel A, Tracz D M, Nonaka L, Ngo T M, Connell S R, and Taylor D E. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet (O)-mediated tetracycline resistance. Antimicrobial Agents and Chemotherapy, vol. 48, no. 9, pp. 3442–3450, 2004.
8. Gonalez Isabel, Katheleen A Grant, Richardson P T, Park S F, and Collins M D. Specific Identification of the Enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by Using a PCR Test Based on the *ceu*E Gene Encoding a Putative Virulence Determinant. Journal Of Clinical Microbiology, 0095-1137/97, 10 Mar. 1997, p. 759–763 Vol. 35, No. 3.
9. Hindawi Publishing Corporation, BioMed Research International, Volume 2013, Article ID 340605, 12 pages: Antimicrobial Resistance Mechanisms among *Campylobacter.* doi.org/10.1155/2013/340605.
10. ISO 10272-1, 2006. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of thermotolerant Campylobacter. international standards organization, Geneva.
11. Kashoma IP, Kumar A, Sanad YM, Gebreyes W, Kazwala RR, Garabed R, Rajashekara G. Phenotypic and genotypic diversity of thermophilic *Campylobacter spp*. in commercial turkey flocks: a longitudinal study. Foodborne Pathog Dis. 2014 Nov; 11(11):850-60. doi: 10.1089/fpd.2014.1794. Epub 2014 Sep 3.
12. Konkel M E, Gray S A, Kim B J, Garvis S G, and Yoon J. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cad* F virulence gene and its products. J. Clin. Microbiol. 37:510–517.
13. Korsak D, Maćkiw E, Rożynek E, Żyłowska M. Prevalence of *Campylobacter spp.* in Retail Chicken, Turkey, Pork, and Beef Meat in Poland between 2009 and 2013. J Food Prot. 2015 May;78 (5):1024-8. doi: 10.4315/0362-028X. JFP-14-353.
14. Lin J, Overbye M L, and Zhang Q J. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrobial Agents and Chemotherapy, vol. 46, no. 7, pp. 2124–2131, 2002.
15. Luangtongkum T, Morishita T Y, Aaron J I, Huang S, Mc Dermott P F, and Zhang Q. Effect of Conventional and Organic Production Practices on the Prevalence and Antimicrobial Resistance of *Campylobacter* spp. in Poultry. Appl Environ Microbiol. 2006 May; 72(5): 3600–3607. doi: 10.1128/AEM.72.5.3600-3607.2006. Ewnetu D, and Mihret A. Prevalence and antimicrobial resistance of Campylobacter isolates from humans and chickens in Bahir Dar, Ethiopia. Foodborne Pathog Dis. 2010 Jun; 7(6):667-70. doi: 10.1089/fpd.2009.0433.
16. Martin S I and Kaye K M. Beta-lactam antibiotics: newer formulations and newer agents. Infectious Disease Clinics of North America, vol. 18, no. 3, pp. 603–619, 2004.
17. Nachamkin I, Allos B M, and Ho T. *Campylobacter species* and Guillain-Barré syndrome. Clin. Microbiol. Rev. 1998.11:555-567.
18. Nayak R, Stewart TM, Nawaz MS. PCR identification of *Campylobacter coli* and *Campylobacter jejuni* by partial sequencing of virulence genes. Mol Cell Probes. 2005 Jun; 19 (3):187-93.
19. Pamela A, Doetkottb O C, Fakhra M K, Loguea C M. Prevalence of the Campylobacter multi-drug efflux pump (*Cme*ABC) in *Campylobacter spp.* Isolated from freshly processed Turkeys. Food Microbiology Volume 23, Issue 5, Pages 453-460.
20. Pérez-Boto D, García-Peña FJ, Abad-Moreno JC, Echeita MA. Antimicrobial susceptibilities of *Campylobacter jejuni*and *Campylobacter coli* strains isolated from two early stages of poultry production. Microb Drug Resist. 2013 Aug; 19 (4):323-30. doi: 10.1089/mdr.2012.0160. Epub 2013 Feb 7.
21. Pfister P, Jenni S, Poehlsgaard J. The structural basis of macrolide-ribosome binding assessed using mutagenesis of 23 S rRNA positions 2058 and 2059. Journal of Molecular Biology, vol. 342, no. 5, pp. 1569–1581, 2004.
22. Poehlsgaard J and Douthwaite S. The bacterial ribosome as a target for antibiotics. Nature Reviews Microbiology, vol. 3, no.11, pp. 870–881, 2005.
23. Quinn, P. J.; Carter, M. E.; Markey, B. and Carter, G. R. Clinical Veterinary Microbiology. 1999. Mosby Publishng Col, London, UK, PP:327-344.
24. Rantsiou K, Lamberti C, and Cocolin L. Survey of *Campylobacter jejuni* in retail chicken meat products by application of aquantitative PCR protocol. International Journal of Food Microbiology 141 (2010) S75–S79.
25. Tajada P, Gomez-Garces J L, Al´os J I, Balas D and Cogollos R. Antimicrobial susceptibilities of *Campylobacter jejuni*and *Campylobactercoli* to 12 𝛽-lactamagents and combinations with 𝛽-lactamase inhibitors. Antimicrobial Agents and Chemotherapy, vol. 40, no. 8, pp. 1924–1925, 1996.

## Viktoria Atanassova, Reich F, Beckmann L and Klein G. Prevalence of Campylobacter *spp*in turkey meat from a slaughterhouse and in turkey meat retail products. FEMS Immunology & Medical Microbiology Volume 49, Issue 1, pages 141–145, February 2007. DOI: 10.1111/j.1574-695X.2006.00180. x.

# WHO, 2015. Campylobacter, fact sheet, reviewed January 2018.http://www.who.int /mediacentre /factsheets/fs255/en/.

1. Zhang Q and Plummer J. Mechanism of antibiotic resistance in Campylobacter,” in Campylobacter. I. Nachamkin, C. M. Szymanski, and M. J. Blaser, Eds., pp. 263–276, American Society for Microbiology, Washington, DC, USA, 2008.

3/21/2018