Assiut University web-site: www.aun.edu.eg

# MOLECULAR DETECTION OF *CAMPYLOBACTER JEJUNI* IN BROILERS WITH REGARDS TO BIOFILM AND VIRULENCE GENES

## HANAA A. MAHMOUD, MOHAMMED W. ABD AL-AZEEM AND HAMS M.A. MOHAMED

Department of Microbiology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

Received: 8 August 2024; Accepted: 5 September 2024

### ABSTRACT

*Campylobacter* is one of the most frequent bacterial foodborne pathogens worldwide. Poultry is the disease's most clinically significant host species. Despite their importance to public health, the virulence factors and mechanisms that drive C. jejuni pathogenicity are poorly understood, and the relationships between these genes and strain origins remain unclear. In this study, we intended to examine the prevalence, virulence and biofilm formation genes, and antibiotic resistance of C. jejuni isolated from cloacal swab samples of commercial chicken in Aswan Governorate, Egypt. Random samples of fresh chickens (n = 103) were collected from different retail markets. To identify the virulence genes (flaA, cdtB, and cadF genes), polymerase chain reaction was employed in conjunction with the 23S rRNA and mapA genes unique to Campylobacter and C. jejuni, respectively. 17 (16.5%) of the 103 samples had positive Campylobacter spp. tests. C. jejuni was present in nine (8.7%) of the positive isolates. In 77%, 100%, and 88.8% of C. jejuni strains, the genes flaA, cdtB, and cadF were detected, respectively. The antibiotic resistance of the C. jejuni isolates was determined via the disc diffusion method and was observed most frequently to ceftazidime (88.9%), ceftriaxone (77.8%), cephalexin (77.8%), erythromycin (66.6%), while low resistance to levofloxacin (11.1%), and chloramphenicol (11.1%) was detected. These findings highlight the high prevalence of Campylobacter in fresh chickens, which is thought to be the main risk factor for domestically obtained campylobacteriosis in Aswan Governorate, Egypt.

Keywords: C. Jejuni; broilers; 23S rRNA; virulence genes; antibiotic resistance

## **INTRODUCTION**

In outbreaks of foodborne illnesses involving chicken meat, *campylobacter* spp. is the most frequently found pathogen (Gourley *et al.*, 2017). The deadly zoonotic illness known as campylobacteriosis, or infection with *Campylobacter* spp., induces gastroenteritis in humans. Eating undercooked poultry meat is one of the biggest risk factors for infection (Freitas and Noronha, 2007).

In accordance with the European Food and Safety Authority (EFSA) and the European Center for Disease Control (ECDC, 2006). The Community Overview Report on Zoonoses, campylobacteriosis continues to

Corresponding author: HANAA A. MAHMOUD E-mail address: hanaaabdelateef.mahmoud@gmail.com Present address: Department of Microbiology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

be the most commonly reported zoonotic disease that affects individuals in the European Union (EU). In addition to the long-term consequences of arthritis. hemolytic uremic disorder, bowel inflammation, functional gastrointestinal abnormalities. and, in extreme cases, Guillain-Barre syndrome, this infection usually presents as acute gastroenteritis.

As reported on <u>http://www.bacterio.net/</u>, the genus *Campylobacter* currently has 66 species and 16 subspecies identified. (Man, 2011; Kaakoush *et al.*, 2015 and Van *et al.*, 2015).

At about 80% of all *Campylobacter* infections, *Campylobacter jejuni* is the species with the largest clinical impact (Whitehouse *et al.*, 2018 and WHO, 2020). *C. jejuni* is susceptible to acidity, freezing, warmth, and normal oxygen concentrations (Nayak, 2012). Relatively common *campylobacter* infections lead to significant medical and financial costs (O'Brien SJ, 2017).

Because of the helical, curved, or S-shaped appearance of these bacteria, the family *Campylobacter* was given its name after the Greek word "curved rod" (Jordan *et al.*, 2001).

According to Gharst, Oyarzabal, and Hussain (2013), *Campylobacter* is a microaerobe that grows best in a temperature range of 35 to 42°C and requires from 2% to 10% oxygen levels (microaerophilic).

During the rearing phase, *C. jejuni* can appear in broiler chicks as early as 14 days of age. By the end of the grow-out period, a low percentage of the birds will have been highly contaminated (EFSA, 2010). A significant amount of *Campylobacter* species are known to be present in the gastrointestinal tract of chickens during processing, especially in the caecum and colon. This is especially the case if the gastrointestinal tract breaks down and the contents are transferred to the skin, further contaminating the meat (Vinueza-Burgos *et al.*, 2017).

Intestinal colonization, the invasion of bacteria into intestinal cells, cytotoxin production, and extra intestinal translocation mechanisms examples are all of Campylobacter infection (Dasti et al., 2010; Tresse et al., 2017). Some virulenceassociated genes have been identified as being responsible for these mechanisms, such as *flaA* and *cadF*, which are responsible for adhesion and colonization, ciaB and virB11, which are involved in host cell invasion, and *cdtA*, *cdtB*, and *cdtC* which are responsible for cytotoxin production (Bolton, 2015).

According to research by Bronnec *et al.* (2016), *C. jejuni* has been shown to form biofilm on a variety of surfaces, including plastic, glass, and steel, at varying oxygen concentrations. As a result, biofilms containing *C. jejuni* pose a serious risk to food safety.

Until now, it is unclear how *C. jejuni's* biofilm formation is regulated at the molecular level. Some of the involved genes include those responsible for cell motility (*flaA*) (Reuter *et al.*, 2010), cell adhesion (*cadF*), quorum-sensing (*luxS*) (Plummer, 2012).

Antibiotic-resistant *Campylobacteria* develop into multidrug resistance bacteria (MDR) (Mansouri *et al.*, 2012). The MDR *Campylobacter* has been and raised worries due to its resistance to quinolones and erythromycin (Ge *et al.*, 2013). These concerns could have a major impact on public health (Iovine, 2013). According to Chai *et al.* (2008), there was a belief that the resistant strain of *Campylobacter* was inherently harder than the sensitive strain.

This study aimed to determine the prevalence of *C. jejuni* species, their virulence and biofilm formation genes, and

their sensitivity to various antimicrobial drugs in the broiler chickens and birds sold in markets in Aswan governorate of Egypt.

### **MATERIALS AND METHOD**

#### **1. Sample collection**

Cloacal swabs (n=103) were taken from broilers in Aswan governorate, Egypt, of 35–40 days old in retail markets. For microbiological analysis, all samples were gathered in sterilized containers and sent to the lab under frigid temperatures.

### 2. Isolation and identification

### 2.1 Isolation step:

Samples were cultured on modified Campylobacter chosen blood-free agar, mCCDA (Oxoid, CM0739B, England) (Bolton et al., 1984) at 42 °C for 48 hours in the microaerophilic environment, after having been enriched in Bolton selectively enriched broth (Oxoid) (FDA et al., 1998) for 24 hours at 10% CO2 at 42°C. Following incubation, staining with Gram's (Gram negative, S-shaped, curved rod) and biochemical testing (catalase, oxidase, and hippurate hydrolysis tests) were used to identify suspicious colonies.

### 2.2 DNA extraction

Fresh cultures of a likely Campylobacter cultivated species that were in a microaerophilic environment for 48 hours at 42°C vielded bacterial DNA. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, GmbH, Germany), following the manufacturer's instructions.

# **2.3** Molecular confirmation of *C. jejuni* isolates and virulence genes:

PCR is used to identify *Campylobacter* and other bacteria focused on the 23S rRNA gene (Wang *et al.*, 2002). The *mapA* was the intended target gene of a PCR that was used to identify *C. jejuni*, according to Eunju & Lee (2009). Table (1) lists the primers acquired from Metabion (Germany) for this project. Using 250-µL PCR reaction tubes, 12.5  $\mu$ L of EmeraldAmp GT PCR master mixes (2x premix), 2  $\mu$ L of 20× the primer mix (1  $\mu$ M for every primer), 5.5  $\mu$ L of PCR graded water, and 5  $\mu$ L of DNA fragments were used for the PCR amplifications. Table (2) contains the cycling parameters for every gene. After staining with ethidium bromide, the generated PCR products were electrophoresed with 1.5% (w/v) agarose in 1× TBE buffer and examined by UV transillumination.

### 3. Antimicrobial susceptibility testing

Following the manufacturer's instructions, according to Finegold and Martin (1982), the conventional disc diffusion method was used on Mueller-Hinton agar mixed with 5% defibrinated sheep blood to assess C. jejuni susceptibility to antibiotics. Azithromycin  $(15\mu g/mL)$ , erythromycin  $(15\mu g/mL)$ , levofloxacin  $(5\mu g/mL)$ , norfloxacin ceftazidime  $(10\mu g/mL)$ ,  $(30\mu g/mL)$ , ceftriaxone  $(30\mu g/mL)$ , cephalexin  $(30\mu g/mL)$ , and chloramphenicol  $(30\mu g/mL)$ where the eight antimicrobials from four classes were employed.

The plates have been incubated for fortyeight hours at 37 °C in a microaerobic environment. The diameter of the zone of inhibition encircling each disc was used to measure the sensitivity of each isolate. The findings were analyzed using family Enterobacteriaceae standards developed by the European Centre for Prevention and Control of Disease (2014) and the Clinical and Lab Standards Institute (CLSI, 2016).

## RESULT

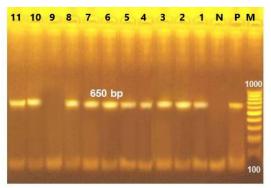
In this investigation, twenty-five (24.3%) suspected *Campylobacter* isolates out of 103 samples gathered from different sources in Aswan governorate, Egypt. The colonies had a smooth, transparent appearance with a regular border. When the colonies were examined under a microscope while still fresh, bacteria with the distinctive motility of *Campylobacter* were found in the midge's

flight. Gram staining, or microscopic immersion observation, verified the presence of spiral or S-curved or Gramnegative bacilli.

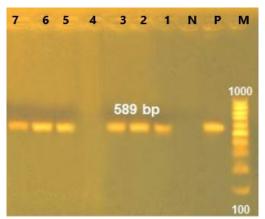
A conventional biochemical method was employed to identify 25 isolates of Campylobacter that were similar. These isolates also showed positive results for hippurate hydrolysis, oxidase, and catalase. Then, using PCR, the similar 25 isolates of C. jejuni were genotypically identified. The isolates were sent for genetic verification Campylobacter and C. jejuni species using amplification with PCR of the 23S rRNA and mapA genes, respectively. Of the examined isolates, 8 (7.8%) tested negative and 17 (16.5%) were recognized as Campylobacter. Table (3) shows that 9 isolates (8.7%) were confirmed to be C. jejuni.

Three critical virulence genes (*flaA*, *cdtB*, and *cadF*) implicated in *C. jejuni* pathogenicity were screened for in all nine molecularly verified *C. jejuni* isolates. Of the nine examined isolates, Table (4) showed that 7 (77.7%), 9 (100%) and 8 (88.8%) tested positive for the *flaA*, *cdtB*, and *cadF* genes, respectively.

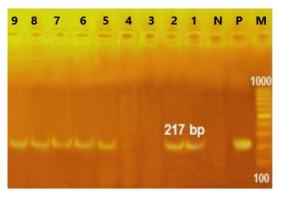
As shown in Table (5), the sensitivity of each of the nine C. jejuni isolates to different antimicrobial medications from different groups was examined. A significant number of C. jejuni were resistant to the cephalosporins group (Ceftazidime, Ceftriaxone, Cephalexin) (81.4%), Erythromycin (66.6%) and Norfloxacin (55.6%). Conversely, the results showed a noteworthy frequency of levofloxacin susceptibility (88.9%), followed by chloramphenicol (66.6%).



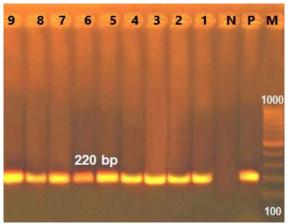
**Figure 1:** Agarose gel electrophoresis of 23S rRNA gene showing bands at 650 bp. Lane M: Marker (DNA ladder 100 bp), Lane P: positive control, Lane N: negative control, Lane (1, 2, 3, 4, 5, 6, 7, 8, 10): positive 23S rRNA gene and Lane (9): negative isolate.



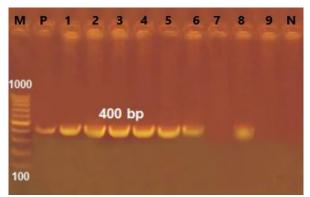
**Figure 2:** Agarose gel electrophoresis of mapA gene showing bands at 589 bp. Lane M: Marker (DNA ladder 100 bp), Lane P: positive control, Lane N: negative control, Lane (1, 2, 3, 5, 6, 7,): positive mapA gene and Lane 4: negative isolate



**Figure 3:** Agarose gel electrophoresis of flaA gene showing bands at 217 bp. Lane M: Marker (DNA ladder 100 bp), Lane P: positive control, and Lane N: negative control, Lane (1, 2, 5, 6, 7, 8, 9): positive *flaA* gene, Lane (3,4): negative isolate,



**Figure 4:** Agarose gel electrophoresis of cdtB gene showing bands at 220 bp. Lane M: Marker (DNA ladder 100 bp), Lane P: positive control, and lane N: negative control, Lane (1, 2, 3, 4, 5, 6, 7, 8, 9): positive cdtB gene.



**Figure 5:** Agarose gel electrophoresis of cadF gene showing bands at 400 bp. Lane M: Marker (DNA ladder 100 bp), Lane P: positive control, Lane (1, 2, 3, 4, 5, 6, 8): positive *cadF* gene, Lane (7, 9): negative isolate, and Lane N: negative control

Target gene	Primer sequence (5'-3')	Length of amplified product	Reference	
<i>C. jejuni</i> mapA	CTA TTT TAT TTT TGA GTG CTT GTG	589 bp	Eunju and Lee, 2009	
	GCT TTA TTT GCC ATT TGT TTT ATT A			
<u>Campylobacter</u>	TATACCGGTAAGGAGTGCTGGAG 650 bp		Wang et al.,	
<u>23S Rrna</u>	ATCAATTAACCTTCGAGCACCG		2002	
<u>FlaA</u>	TCCAAATCGGCGCAAGTTCA	217 bp	Zheng et al.,	
	TCAGCCAAAGCTCCAAGTCC		2006	
<u>cdtB</u>	CAGAAAGCAAATGGAGTGTT	220 bp	Nahar and	
	AGCTAAAAGCGGTGGAGTAT		Bin Rashid, 2018	
CadF	TTG AAG GTA ATT TAG ATA TG	400 bp	Al Amri et	
	CTA ATA CCT AAA GTT GAA AC		al., 2007	

# Table 1: Oligonucleotide primers sequences.

**Table 2:** Cycling conditions of the primers during cPCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
C. jejuni	94°C	94°C	55°C	72°C	35	72°C
	5 min.	30 sec.	45 sec.	45 sec.		10 min.
Campylobacter	94°C	94°C	55°C	72°C	35	72°C
23S rRNA	5 min.	30 sec.	40 sec.	45 sec.		10 min.
FlaA	94°C	94°C	55°C	72°C	35	72°C
	5 min.	30 sec.	30 sec.	30 sec.		7 min.
CdtB	94°C	94°C	51°C	72°C	35	72°C
	5 min.	30 sec.	40 sec.	45 sec.		10 min.
CadF	94°C	94°C	49°C	72°C	35	72°C
	5 min.	30 sec.	40 sec.	40 sec.		10 min.

Table 3: Occurrence of 0	<i>Campylobacter</i> in	the examined	samples using PCR:
--------------------------	-------------------------	--------------	--------------------

	+Ve Campylobacter spp.		+Ve C. Jejuni		Other Spp.	
samples	No.	%	No.	%	No.	%
103	17	16.5%	9	8.7%	8	7.8%

No. of C. Jejuni isolates	flaA gene		cdtB gene		cadF gene	
C. Jejuin isolates	No.	%	No.	%	No	%
9	7	77.7%	9	100%	8	88.8%

Table 4: Occurrence of some	virulence	genes in	Campylobacter	isolates	obtained	from the
examined samples						

Classes of	Antimicrobial agents	Re	<b>Resistant strains</b>				
antibiotics	C	S	Ι	R	strains to all antibiotic classes		
Macrolides	Erythromycin(15µg/mL) Azithromycin(15µg/mL)	3(33.4%) 5(55.6%)	0(0%) 1 (11.1%)	6(66.6%) 3(33.3%)	50%		
Fluoroquinolones	Levofloxacin (5µg/mL) Norfloxacin (10µg/mL)	7(77.8%) 3(33.3%)	1 (11.1%) 2(22.2%)	1(11.1%) 4(44.5%)	33.3%		
Cephalosporins	Ceftazidime (30µg/mL) Ceftriaxone (30µg/mL) Cephalexin (30µg/mL)	0(%) 1(11.1%) 2(22.2%)	1(11.1%) 1(11.1%) 0(0%)	8(88.9%) 7(77.8%) 7(77.8%)	81.4%		
Quinolones	Chloramphenicol (30µg/mL)	6(66.6%)	2(22.3%)	1(11.1%)	11.1%		

Zone diameter of Erythromycin, Azithromycin, levofloxacin, Norfloxacin, Ceftazidime, Ceftrixone, Cephalexine and Chloramphenicol were recommended by the (CLSI-2016) for Enterobacteriaceae and European Centre for Disease Prevention and Control (2014). S, susceptible; I, intermediate; R, resistant

# DISCUSSION

Even in wealthy countries, *C. jejuni* is considered one of the most common and dangerous foodborne pathogens. It induces serious gastroenteritis with bacteria in humans who consume contaminated food, especially poultry and related products (Eurosurveillance Editorial Team, 2012). Using both conventional and polymerase chain reaction approaches, we were able to determine the overall prevalence of *campylobacter* species in the analyzed samples. These results came out to be 24.3% and 8.7%, respectively.

This prevalence may be related to the intestinal tract of the chicken, specifically the colon and caecum, which are thought to be regions of tropism for a variety of species of *Campylobacter* (Jokinen *et al.*, 2011).

As to Hansson *et al.* (2018), the prevalence of *campylobacter* differs depending on the nation. One cause of these changes is C.

jejuni mutations, because of intragenomic processes as well as strain-specific genetic exchange, C. jejuni possesses a high level of genetic diversity. Through C. jejuni genome sequencing, homopolymeric tractbased hypervariable transcripts have been found, most of the hypervariable sequences that have been described are located in regions that encode proteins that are involved in the synthesis or modification of surface-accessible carbohydrate structures capsule, lipooligosaccharide such the (LOS), or flagellum. Change in these structures is caused by a variety of causes, including frame shifts, points of mutation, duplication of genes and suppression, and phase variation.

The plasmids of *Campylobacter* species play a major role in determining their capacity to adapt and turn harmful. Research has identified many plasmid families, including pVir-like and pTet-like; these families each contain distinct relaxases or replicon types, and their effects may have an impact on the virulence and survival of bacteria (van Vliet *et al.*, 2021; Hull *et al.*, 2023). Additionally, plasmids aid in the horizontal transfer of genes, which transmits genetic material and traits quickly and increases the adaptability of populations of *Campylobacter* spp. The presence of plasmids containing virulence factors emphasizes their importance in the potential for infections of these bacteria.

According to Ica al. (2012),et Campylobacter may be able to live in biofilms outside of stress response mechanisms in natural environmental Campylobacter biofilms can settings. develop in the plumbing and water supply systems of poultry husbandry establishments food processing and factories, in addition to the gastrointestinal tracts of poultry. Poultry can then either directly or indirectly introduce these biofilms into the human food chain (Siringan et al., 2011).

Molecular understanding of *C. jejuni* biofilm formation is still in its infancy although there is evidence for the role of flagella and gene regulation in biofilm formation (Reeser *et al.*, 2007 and Svensson *et al.*, 2009), For instance, genes involved in stress response and flagella synthesis (*flaA*) can control the development of *C. jejuni* biofilms (Kalmokoff *et al.*, 2006; Reeser *et al.*, 2007 and Svensson *et al.*, 2007). Reeser *et al.* (2007) discovered that a *C. jejuni* flagella knockout mutant ( $\Delta$ flaAB) produced a notably decreased level of biofilm in contrast to its wild-type counterpart.

Of virulence genes identified in isolates of *Campylobacter jejuni* found in poultry, the *flaA* genes, which are necessary for flagellar motility, were present in 77.7% of *C. jejuni* isolates. Our results are entirely in line with earlier research conducted in Egypt. As it affects the mobility, adhesion, and invasion of host intestinal epithelial cells and biofilm

formation, the *flaA* gene is a virulent marker in *C. jejuni* strains.

*CadF* is one of the reference virulence genes that encode proteins involved in the attack and attachment of *C. jejuni* (Elmali & Can, 2019), and this gene is present at a high prevalence in *C. jejuni* isolates (Andrzejewska *et al.*, 2015). According to Melo *et al.* (2017), the presence of the genes *flaA* and *cadF* in *Campylobacter* strains indicated a high potential for biofilm formation.

*CadF* gene was detected in 8 (88.8%) of *Campylobacter* isolates. These results are similar to data previously reported by other authors Datta *et al.* (2003); Rozynek *et al.* (2005); Krutkiewicz and Klimuszko (2010). A higher result (100%) was obtained by da Silva *et al.* (2021), while Ghoneim *et al.* (2020) and Abbas *et al.* (2021) recorded a lower result (20.58%) and (20.5%), respectively.

Another significant component of *Campylobacter* is the *CDT* complex, which codes for cytolethal distending toxin, with *cdtB* serving as the catalytic site. *CDT* is composed of three subunits (CdtA, CdtB and CdtC), the catalytic subunit CdtB, which triggers cell cycle arrest and causes the intestinal epithelium and immune cells to undergo apoptosis, whereas *CdtA*, and *CdtC* are binding proteins for delivering CdtB into target cells (Jain et al., 2008). 100% of the isolates examined in this investigation had the *cdtB* identified, which is in line with earlier findings by Asakura et al. (2010) and Jribi et al. (2017).

Antibiotics are profusely administered for therapeutic and prophylaxis purposes in the veterinary field (Dandachi *et al.*, 2018). In recent years, disinfectants have been used with carelessness leading to the adaptation of bacteria and augmenting the spread of resistant bacteria.

Antimicrobial Nowadays, resistance, especially to fluoroquinolone (ciprofloxacin) and macrolides (erythromycin), emerged *Campylobacter* has in (Lehtopolku et al., 2011). The use of tetracycline while rearing farm animals has been reviewed in recent years because of its growth-promoting properties, The addition of a subtherapeutic dose of chlortetracycline in livestock rations positively affects the rate of growth and feed utilization of young chickens. Therefore, a significant increase in antibiotic resistance has been observed in Campylobacter isolates recovered from chickens (EFSA, 2012).

In the present study, very high resistance rates (81.4%) to cephalosporins were detected. Similar results have been found by varela *et al.* (2007), Griggs *et al.* (2009) and Giacomelli *et al.* (2014). On the contrary, Raeisi *et al.* (2017) and Ghoneim *et al.* (2020) reported high susceptibility of *C.jejuni* to cephalosporins.

Meanwhile, the isolates were susceptible to fluoroquinolones (Levofloxacin) and chloramphenicol, similar to other studies Barco *et al.* (2010), De Cesare *et al.* (2008), Parisi *et al.* (2007), Pezzotti *et al.* (2003). However, our results contrast those of Mackiw *et al.* (2012) Zhu *et al.* (2006).

The strategies adopted to limit antimicrobial resistance (AMR) will vary globally, particularly across countries with different degrees of wealth and development. The most effective intervention will likely depend on prevailing conditions. For example, highincome countries and low-income countries can differ in antimicrobial resistance patterns, antimicrobial use practices, access to healthcare services (human and animal), sanitation and infrastructures regulation (Collignon et al., 2018 and Caudell et al., 2017).

# CONCLUSION

This study provided sufficient information on the presence of *Campylobacter* in chickens, with the assumption that fresh birds are the main source of campylobacteriosis.

In addition, customers run the risk of contracting *Campylobacter* infections due to the virulent features present in *C. jejuni* isolates and the bacteria's strong resistance to both cephalosporins and macrolides.

The aforementioned results underscore the significance of instituting hygienic protocols on the farm and controlling the number of *Campylobacter* during the processing of carcasses. Additionally, it is imperative to establish a proficient system for managing *Campylobacter* infections in chickens and curtailing the usage of antibiotics in the poultry industry.

Our research leads us to the conclusion that PCR is essential for the identification of numerous bacteria. PCR is a significant and widely utilized technology that has many uses in medical and biological research labs today.

# REFERENCES

- Abbas, S.G.; Karmi, K.; Mubarak, A.G. and Youseef, A.G. (2022): Prevalence and Virulence Genes Profile of Zoonotic *Campylobacter* species in Chickens and Human in Aswan Governorate: International Journal of Veterinary Sciences, 5(4): 15-32, 2022 and phylogeny in Ireland. PloS One 2019; 14: 88e219.
- Andrzejewska, M.; Szczepańska, B.; Śpica, D. and Klawe, J.J. (2015): Trends in the occurrence and characteristics of Campylobacter jejuni and Campylobacter coli isolates from poultry meat in Northern Poland. Food Control, 51, 190-194.

http://dx.doi.org/10.1016/j. foodcont.2014.11.014

- Asakura, H.; Yamamoto, S.; Tachiban, M. and Yoshimura, M. (2010): Studies on Efficacy of Freezing on the Reduction of *Campylobacter jejuni* in Chicken Meat. Japanese Journal of Food Microbiology, 32(3):159-166.
- L.: Lettini A.A: Pozza, Barco, M.C.D.; Ramon, E.; Fasolato, M. and Ricci, A. (2010): Fluoroquinolone Resistance Detection in *Campylobacter* coli and Campylobacter *jejuni* by Luminex<sup>®</sup> xMAP<sup>TM</sup> Technology Foodborne Pathogens and Disease. https://doi.org/10.1089/fpd.2009.0
- Bolton, D.J. (2015): Campylobacter virulence and survival factors. Food Microbiol. 48: 99–108. doi:10.1016/j.fm.2014.11.017.PMID: 25790997.
- Bolton, F.J.; Hutchinson, D.N. and Coates, D. (1984): Blood-free selective medium for the isolation of *Campylobacter jejuni* from feces. J. Clin. Microbiol., 19, 169 – 171.
- Bronnec, V.; Turoňová, H.; Bouju, A.; Cruveiller, S.; Rodrigues, R. and Demnerova. *K*. et al. (2016): Adhesion, biofilm formation. genomic features and of Campylobacter jejuni Bf, an atypical strain able to grow under aerobic conditions. Front. Microbiol. 7. 1002. 10.3389/fmicb.2016.01002
- Brown, H.L.; Hanman, K.; Reuter, M.; Betts, R.P. and van Vliet, A.H.M. (2015): Campylobacter jejuni biofilms contain extracellular DNA and are sensitive to DNase I treatment, 699–11 Front. Microbiol. 6. <u>https://doi.org/10.3389/fmicb.</u> 2015.00699.
- C. VinuezaBurgos; M. Wautier; D. Martin y; M. Cisneros; V.I. Damme and L. De Zutter (2017): Prevalence, antimicrobial resistance and genetic diversity of Campylobacter

*coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age

*Caudell, MA. et al. (2017):* Antimicrobial Use and Veterinary Care among Agro-Pastoralists in Northern Tanzania. *PLoS ONE*. 2017;12: e0170328.

doi: 10.1371/journal.pone.0170328.

- Chai, L.C.; Fatimah, A.B.; Ghazali, F.M.; Lee, H.Y.; Tunung, R. and Shamsinar, A.T. (2008): Biosafety of Campylobacter jejuni from raw vegetables consumed as Ulam with reference to their resistance to antibiotics. Int Food Res J., 15: 125-34.
- Collignon, P.; Beggs, JJ.; Walsh, TR.; Gandra, S. and Laxminarayan, R. Anthropological (2018): and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. Lancet Planet. Health. 2018; 2:e398–e405. doi: 10.1016/S2542-5196(18)30186-
- Da Silva, W.P.; Lopes, G.V.; Ramires, T. and Fiorentini, A.M. (2021): Virulence factors of foodborne pathogen Campylobacter jejuni: Elsevier Microbial Pathogenesis
- Dandachi, I.; Sokhn, E.S.; Dahdouh, E.A.; Azar, E.; El-Bazzal, B. and Rolain, J.M. et al. (2018): Prevalence and characterization of multi-drugresistant gram-negative bacilli isolated from Lebanese poultry: a nationwide study. Front. Microbiol. 9:550.

10.3389/fmicb.2018.00550

- Dasti, J.I.; Tareen, A.M.; Lugert, R.; Zautner, A.E. and Gross, U. (2010): Campylobacter jejuni: a brief overview on pathogenicity associated factors and disease-mediating mechanisms. Int. J. Med. Microbiol. 300: 205–211. doi:10.1016/j.ijmm. 2009.07. 002. PMID:19665925
- Dasti, J.I.; Tareen, A.M.; Lugert, R.; Zautner, A.E. and Gross, U. (2010): Campylobacter jejuni: a brief

overview on pathogenicity associated factors and disease-mediating mechanisms. Int. J.

- Datta, S.; Niwa, H. and Itoh, K. (2003): Prevalence of 11 pathogenic genes of Campylobacter jejuni by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. Journal of Medical Microbiology 52, 345–348
- De Cesare, A.; Parisi, A.; Bondioli, V.; Normanno, G. and Manfreda, G. (2008): Genotypic and phenotypic diversity within three Campylobacter populations isolated from broiler ceca and carcasses. Poultry Science, 87 : 2152 – 2159. doi: 10.3382/ps.2007-00441
- EFSA (European Food Safety Authority), (2010): Analysis of the baseline survey on the prevalence of Campylobacter in broiler batches and of Campylobacter and Salmonella on broiler carcasses in the EU, 2008-Part A: Campylobacter and prevalence estimates. Salmonella EFSA J. 8:1503. https://doi.org/ 10.2903/j.efsa.2010.1503
- *EFSA*, (2012): The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. *Euro Surveilliance*, 10.3: 2598. <u>https://doi.org/10.2903/j.efs</u>
- *EFSA. (2007):* The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European union. The EFSA J 2007;130:
- *Elmali, M. and Can, H.Y. (2019):* Antimicrobial susceptibility and virulence-associated genes in *Campylobacter* isolates from milk and wastewater in Hatay, Turkey. *Ciência Rural*, 49(5), 1-8. http:// dx.doi.org/10.1590/0103-8478cr20180227.

- *Eunju, S. and Lee, Y. (2009):* Comparison of Three Different Methods for *Campylobacter* Isolation from Porcine Intestines. J. Microbiol. Biotechnol., 19(7): 647-650.
- Eurosurveillance Editorial Team. (2012): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. Eurosurveillance, 17(10), 1. Retrieved from http://www.eurosurveillance.org/Vie wArticle.aspx?ArticleId=20113

http://www.eurosurveillance.org/Vie wArticle.aspx?ArticleId=20113

- FDA, Hunt, J.M. and Abeyta, C.T.T. (1998): FDA Bacteriological Analytical Manual. Chapter 7, *Campylobacter*,. 8th edition (revision A), 23 pages.
- Finegold, S.M. and Martin, W.J. (1982): Diagnosis microbiology, 16h Ed. Th C.V. MosbyCo. St. Louis Toronto London.
- Freitas, J.A. and Noronha, G.N. (2007): Ocorrência de Campylobacter Spp. em carne e miúdos de frango expostos ao consumo em Belém, Pará. Arquivo Brasileiro De Medicina Veterinária E Zootecnia, v.59(3), pp. 813-815. DOI: 10.1590/S0102-09352007000300038.
- Ge, B.; Wang, F.; Sj'lund-Karlsson, M. and McDermott, P.F. (2013): Antimicrobial resistance in Campylobacter: susceptibility testing methods and resistance trends. J Microbiol Methods ., 95: 57-67.
- Gharst, G.; Oyarzabal, O.A. and Hussain, S.K. (2013): Review of Current Methodologies to Isolate and Identify *Campylobacter* spp. from Foods. Journal of Microbiological Methods, 95, 84-92. <u>https://doi.org/10.1016/</u> j.mimet.2013.07.014
- Ghoneim, N.; Sabry, M.; Ahmed, ZS. and Elshafiee, E. (2020): Campylobacter Species Isolated from Chickens in Egypt: Molecular Epidemiology and

Antimicrobial Resistance.Pakistan J. Zool.,pp1-10,2020.

- Giacomelli, M.; Salata, C.; Martini, M.; Montesissa, C. and Piccirillo, A. (2014): Antimicrobial resistance of Campylobacter jejuni and Campylobacter coli from poultry in Italy. Microb. Drug Resist. 2014; 20:181–188
- Gourley, C.R.; Negretti, N.M.; Konkel, M.E. (2017): The food-borne pathogen Campylobacter jejuni depends on the AddAB DNA repair system to defend against bile in the intestinal environment. Sci. Rep. 7:14777. doi: 10.1038/s41598-017-14646-9, PMID.
- Griggs, D.J.; Peake, L.; Johnson, M.M.; Ghori, S.; Mott, A. and Piddock, L.J. (2009): Beta-lactamase-mediated beta-lactam resistance in Campylobacter species: prevalence of Cj0299 (blaOXA-61) and evidence for a novel beta-Lactamase in C. jejuni. Antimicrob. Agents Chemother. 53 3357–3364. 10.1128/AAC.01655-08
- Hansson, I.; Sandberg, M.; Habib, I.; Lowman, R. and Olsson, E.E. (2018): Knowledge gaps in control of Campylobacter for prevention of campylobacteriosis. Transbound. Emerg. Dis. 2018; 65:30–48.
- Hull, D.M.; Harrel, E.; Harden, L. and Thakur, S. (2023): Detection of resistance and virulence plasmids in Campylobacter coli and coli and Campylobacter jejuni isolated from North Carolina food animal production, 2018-2019. Food Microbiol. 116:104348. doi: 10.1016/j.fm.2023.104348, PMID
- Ica, T.; Caner, V.; Istanbullu, O.; Nguyen, H.D.; Ahmed, B.; Call, D.R. and Beyenal, H. (2012): Characterization of mono- and mixed-culture Campylobacter jejuni biofilms. Appl. Environ. Microbiol. 78, 1033–1038.

https://doi.org/10.1128/AEM.07364-11.

- Iovine, N.M. (2013): Resistance mechanisms in Campylobacter jejuni. Virulence, 4: 230-240.infect. Microbiol. 2:22. doi: 10.3389/fcimb.2012.00022
- Jain, D.; Prasad, K.N.; Sinha, S. and Husain, N. (2008): Differences in virulence attributes between cytolethal distending toxin positive and negative *Campylobacter jejuni* strains. J. Med. Microbiol. 57(Pt 3), 267–272. doi: 10.1099/ jmm.0.47317-0.
- Jokinen, C.; Edge, T.A.; Ho, S.; Koning, W.; Laing, C.; Mauro, W.; Medeiros, D.; Miller, J.; Robertson, W.; Taboada, E.; Thomas, J.E.; Topp, E.; Ziebell, *K*. and Gannon. V.P.(2011): Molecular subtypes of Campylobacter spp., Salmonella enterica. and *Escherichia* coli O157:H7 isolated from faecal and surface water samples in the Oldman River watershed, Alberta, Canada. *Water* Research, 45(3), 1247-1257. http://dx.doi.org/10.1016/j.watres.20 10.10.001 PMid: 20971491.

10.10.001 PMid: 20971491. <u>»</u> http://dx.doi.org/10.1016/j.watres.20

10.10.001

- Jribi, H.; Sellami, H.; Mariam, S.; Smaoui, S.; Ghorbel, A.; Hachicha, S. and Gdoura, R. (2017): Isolation and Identification of Camylobacter spp. from Poultry and Poultry By-Products in Tunisia by Conventional Culture Method and Multiplex Real-Time PCR. Journal of Food Protection, 80: 1623-1627.
- Kaakoush, N.O.; Castaño-Rodríguez, N.; Mitchell, H.M. and Man, S.M. (2015): Global epidemiology of Campylobacter infection. Clin. Microbiol. Rev. 28, 687–720. doi: 10.1128/CMR.00006-15
- Kalmokoff, M.; Lanthier, P.; Tremblay, T.-L.; Foss, M.; Lau, P.C.; Sanders, G.;

Austin, J.; Kelly, J. and Szymanski, C.M. (2006): Proteomic analysis of *Campylobacter jejuni* 11168 biofilms reveals a role for the motility complex in biofilm formation. J. Bacteriol. 188, 4312–4320. <u>https://doi.org/</u> 10.1128/JB.01975-05.

- Krutkiewicz, A. and Klimuszko, D. (2010): Genotyping and PCR detection of potential virulence genes in *Campylobacter jejuni and Campylobacter coli* isolated from different sources in Poland. Folia Microbiologica 55, 167–175.
- Lehtopolku, M.; Kotilainen, P.; Haanpera-Heikkinen. *M*.: Nakari. *U.M.*: *M.L.*; Hanninen, Huovinen, *P*.: Siitonen, A.; Eerola, E.; Jalava, J. and Hakanen, A.J. (2011): Ribosomal mutations as the main cause of macrolide resistance in *Campylobacter* jejuni and *Campylobacter* coli. Antimicrob. Agents Chemother., 55: 5939–5941. https://doi.org/10.1128/AAC.00314-11

https://doi.org/10.1128/AAC.48.9.34 42-3450.2004

- Mackiw, E.; Korsak, D.; Rzewuska, K.; Tomczuk, K. and Rozynek, E. (2012): Antibiotic resistance in Campylobacter jejuni and Campylobacter coli isolated from food in Poland. Fd. Contr., 23: 297-301. <u>https://doi.org/</u> 10.1016/j.foodcont.2011.08.022
- Man, S.M. (2011): The clinical importance of emerging *Campylobacter* species.
- Mansouri, N.L.; Saleha, A.A. and Wai, S.S. (2012): Prevalence of multidrug resistance Campylobacter jejuni and Campylobacter coli in chickens slaughtered in selected markets, Malaysia. Trop Biomed., 29: 231-8.
- *Med. Microbiol.* 300: 205–211. doi:10.1016/j.ijmm.2009.07.002. PMID: 19665925
- Melo, R.T.; Mendonça, E.P.; Monteiro, G.P.; Siqueira, M.C.; Pereira, C.B.; Peres, P.A.B.M.; Fernandez, H. and

*Rossi, D.A. (2017):* Intrinsic and extrinsic aspects on Campylobacter jejuni biofilms. Front. Microbiol. 8, 1332–1346. https://doi.org/10.3389/fmicb.2017.01332.

- Nayak, T. (2012): In: Bad Bug book: Foodborne Pathogenic Microorganisms and Natural Toxins. Lampel KA, editor. Food and Drug Administration; 2012. pp. 14– 17
- O'Brien, SJ. (2017): The consequences of Campylobacter infection. Curr. Opin. Gastroenterol. 2017; 33:14– 20.doi: 10.1097/mog.00000000000 0329.
- Parisi. A.; Lanzilotta, SG.; Addante, N.; Normanno, G.; Di Modugno, G. and Dambrosio, A. (2007): Prevalence, molecular characterization and antimicrobial resistance of thermophilic campylobacter isolates from cattle, hens, broilers and broiler meat in south-eastern Italy. Vet Res Commun 31:113-23
- Pezzotti, G.; Serafin, A.; Luzzi, I.; Mioni, R.; Milan, M. and Perin, R. (2003): Occurrence and resistance to antibiotics of campylobacter jejuni and campylobacter coli in animals and meat in northeastern Italy.
- Plummer, P.J. (2012): LuxS and quorumsensing in *Campylobacter*. Front. Cell. PMID:25790997
- Raeisi, M.; Khoshbakht, R.; Ghaemi, EA.; Bayani, *M*.: Hashemi, *M*.: Seyedghasemi, NS. and Shirzad-Aski, H. (2017): Antimicrobial resistance and virulence-associated genes of *Campylobacter* spp isolated from raw milk, fish. poultry. and red meat. Microb. Drug Resist. 2017;23:925-933.
- Redondo, N.; Carroll, A. and Mc Namara, E. Molecular characterization of *Campylobacter* causing human clinical infection using wholegenome sequencing: virulence, antimicrobial resistance

- Reeser, R.J.; Medler, R.T.; Billington, S.J.; Jost, B.H. and Joens, L.A. (2007): Characterization of campylobacter jejuni biofilms under defined growth conditions. Appl. Environ. Microbiol. 73, 1908–1913. <u>https://doi.org/</u> 10.1128/AEM.00740-06.
- Reuter, M.; Mallett, A.; Pearson, B.M.; Van Vliet, A.H.M. (2010): Biofilm formation by Campylobacter jejuni is increased under aerobic conditions. Appl. Environ. Microbiol. 76, 2122– 2128.

https://doi.org/10.1128/AEM.01878-09.

- Rozynek, E.; Dzierzanowska-Fangrat, K.; Jozwiak, P.; Popowski, J.; Korsak, D. and Dzierzanowska, D. (2005): Prevalence of potential virulence markers in Polish Campylobacter jejuni and Campylobacter coli isolates obtained from hospitalized children and from chicken carcasses. Journal of Medical Microbiology 54, 615–619
- Siringan, P.; Connerton, P.L.; Payne, R.J.H. and Connerton, I.F. (2011): Bacteriophage-mediated dispersal of *Campylobacter jejuni* biofilms. Appl. Environ. Microbiol. 77, 3320–3326. <u>https://doi.org/10.1128/AEM.02704-10</u>.
- Svensson, S.L.; Davis, L.M.; MacKichan, J.K.; Allan, B.J.; Pajaniappan, M.; Thompson, S.A. and Gaynor, E.C. (2009): The CprS sensor kinase of the zoonotic pathogen Campylobacter jejuni influences biofilm formation and is required for optimal chick colonization. Mol. Microbiol. 71, 253–272.

https://doi.org/10.1111/j.1365-

<u>2958.2008.06534.x</u>.

Svensson, S.L.; Pryjma, M. and Gaynor, E.C. (2014): Flagella-mediated adhesion and extracellular DNA release contribute to biofilm formation and stress tolerance of *Campylobacter jejuni*. PLoS One 9, e106063. https://doi.org/10.1371/journal. pone.0106063.

Tresse, O.; Alvarez-Ordonez, A. and Connerton, I.F. (2017): Editorial: about the foodborne pathogen Campylobacter. Front. Microbiol.8: 1908. doi:10.3389/fmicb.2017.01908. PMID: 29067004.

Tresse, O.; Alvarez-Ordonez, A. and Connerton, I.F. (2017): Editorial: about the foodborne pathogen Campylobacter. Front. Microbiol. 8: 1908. doi:10.3389/fmicb.2017.01908.

PMID: 29067004.

- Van der Stel, A.-X. M.; van Mourik, A.; Lanniewski, P.; van der Putten, J.P.M.; Jagusztyn-Krynicka, E.K. and Wosten, M.M.S.M. (2015): The Campylobacter jejuni RacRS twocomponent system activates the glutamate synthesis by directly upregulating g-
- *Glutamyltranspeptidase (GGT):* Front. Microbiol. 6:3389/fmicb.2015.00567:567. doi: 10.3389/fmicb.2015.00567
- Van Vliet, A.H.M.; Charity, O.J. and Reuter, M. (2021): A Campylobacter integrative and conjugative element with a CRISPR-Cas9 system targeting competing plasmids: A history of plasmid warfare: *Microb. Genom.* 7:000729. doi: 10.1099/mgen.0.000729, PMID
- Varela, N.P.; Friendship, R. and Dewey, C. (2007): Prevalence of resistance to 11 antimicrobials among *Campylobacter coli* isolated from pig on 80 growerfinisher farms in Ontario. Can. J. Vet. Res. 71, 189–194.
- Wang, G.; Clark, C.G.; Taylor, T.M.; Pucknell, C.; Barton, C.; Price, L.; Woodward, D.L. and Rodgers, F.G. (2002): Colony multiplex PCR assay for identification and differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus

subsp. fetus. J Clin Microbiol., 40(12): 4744-4747.

Whitehouse, CA.; Zhao, S. and Tate, H. (2018): In: Advances in Applied Microbiology. Sariaslani S, Gadd GM, editors. Academic Press; 2018. pp. 1–47. World Health Organization. (WHO, Geneva, Switzerland, 2020).

Zhu, J.; Zhang, Y.; Hua, X.; Hou, J. and Jiang, Y. (2006): Antibiotic resistance in Campylobacter. Rev. med. Microbiol., 17:107121. <u>https://doi.or</u> g/10.1097/MRM.0b013e3280c4d106

# الكشف الجزيئي لميكروب الكامبيلوباكتر جيجناي المعزول من دواجن التسمين فيما يتعلق بالجينات الخاصة بالضراوة وتكوين الأغشية الحيوية

## هناء عبد اللطيف محمود ، محمد وائل عبد العظيم ، همس محمد

Email: <u>hanaaabdelateef.mahmoud@gmail.com</u> Assiut University web-site: <u>www.aun.edu.eg</u>

يعد ميكروب الكامبيلوباكتر من أكثر الميكروبات المسببة للامراض البكتيرية الغذائية شيوعا في جميع أنحاء العالم، والدواجن هي أكثر الأنواع المضيفة. وعلى الرغم من أهمية ميكروب الكامبيلوباكتر جيجناي في المرض من الناحية السريرية ، إلا أن عوامل الضراوة والآليات التي تدفع مرضية وأصول السلالات لا تزال غير واضحة.

ولذلك قمنا في هذه الدر اسة بفحص انتشار جينات الضر اوة ومقاومة المضادات الحيوية للكامبيلوباكتر جيجناي والتي تم عزلها من عينات تم أخذها من منافذ الدواجن التجارية في محافظة أسوان بمصر.

تم جمع عينات عشوائية من الدواجن الحية من محلات التجزئة للحوم الداجنة و عددها ١٠٣ عينة بهدف تحديد جينات الضراوة (الجينات flaA و cdtB و cdtB و cddF )، تم استخدام تفاعل البلمرة المتسلسل بالإضافة إلى جينات SrRNA و 23S الجوب الخاصة لكامبيلوباكتر وكامبيلوباكتر جيجناي، على التوالي. كانت ١٧ عينة (٦٦,٥٪) من العينات الـ ١٠٣ إيجابية لفحص سلالات الكامبيلوباكتر وكانت سلالة الكامبيلوباكتر جيجناي موجودة في تسع عينات (٨,٧٪) من السلالات الإيجابية. تم الكشف عن الجينات *flaA و cdtB و cdtB في ٧٧*% و ١٠٠ مرهم من سلالات كامبيلوباكتر جيجناي، على التوالي.

كذلك قمنا بإجراء اختبار حساسية لثمانية أنواع من المضادات الحيوية لتسع سلالات كامبيلوباكتر جيجناي، أظهرت السلالات مقاومة للسيفتازيديم بنسبة (۸۸٫۹٪) والسيفترياكسون بنسبة (۷۷٫۸٪) والسيفاليكسين بنسبة (۷۷٫۸٪) والإريثرومايسين بنسبة (۲٦٫٦٪) والنور فلوكساسين بنسبة (٤٤٫٤٪) والأزيثرومايسين بنسبة (۳۳٫۳٪) والليو فلوكساسين بنسبة (۱۱٫۱٪) والكلور امفينيكول بنسبة (۱۱٫۱۰%)

كانت أغلب سلالات C. jejuni التي تم العثور عليها مقاومة للسيفالوسبورين والمكروليدات، وكان هناك انتشار مرتفع للحساسية للكلور امفينيكول. تدعم هذه النتائج الحاجة الملحة لرقابة سلامة الغذاء وتبرز الانتشار العالي لكامبيلوباكتر في الدواجن الطازجة، والتي يُعتقد أنها العامل الرئيسي في الإصابة بمرض كامبيلوباكتر في المنازل في محافظة أسوان، مصر.