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# OVERVIEW ON THE INHIBITORY EFFECT OF GREEN TEA EXTRACT NANOPARTICLES ON CAMPYLOBACTER ISOLATED FROM CHICKEN MEAT

MOHAMED NABIL[1], ENAS, A.M. ALI[1], NESMA M.G. AHMED[2],
RABAB RAWASH[1], OLA FATHY[1], LAMYA, A.F. ATTEYA[3],
REHAB GAAFAR[1] AND NARIMAN ABD ELHADY[1]

[1]Department of Food Hygiene, Animal Health Research Institute (AHRI) – Benha Lab., Agriculture Research Center (ARC), Egypt.
[2]Department of Bacteriology, Animal Health Research Institute (AHRI) – Benha Lab., Agriculture Research Center (ARC), Egypt.
[3]Department of Virology, Animal Health Research Institute (AHRI) – Benha Lab., Agriculture Research Center (ARC), Egypt.

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## ABSTRACT

*Campylobacter species* are significant foodborne pathogens commonly found in chicken meat. Therefore, the current study aimed to determine the prevalence of *Campylobacter species* in fifty varied samples of raw fresh chicken meat collected from random markets located in Qalubiya governorate, Egypt. Results revealed that *Campylobacter species* was detected in 38.0% of the examined samples, where the prevalence of *C. jejuni* and *C. coli* was 30.0% and 8.0%, respectively. Furthermore, virulence of the isolated *C. jejuni* was determined molecularly; where *cdt*A and *cdt*B genes were positively-detected in 100% and 40.0% of the examined isolates, respectively. For the significance and welling to reduce its prevalence in chicken meats, the antibacterial effect of green tea extract (GTE) nanoparticles, 250 and 500 ppm (wt/wt), was investigated against experimentally inoculated chicken fillet with *C. jejuni* during refrigeration storage. Results revealed significant enhancements with elongation in the organoleptic acceptability scores in the treated samples in relation to the control group. Physical enhancement effect of GTE was concentration dependent, where the treated chicken fillet with 500 ppm GTE preserved its acceptability up to ten days of storage. Additionally, significant reductions in *C. jejuni* count were recorded in the treated samples, where higher GTE concentration revealed a higher reduction in percentage up to twelve days of refrigeration storage. In conclusion, GTE showed a potential anti-campylobacter effect, with promising use in chicken meat preservation.

***Keywords:*** Campylobacteriosis, Nanoparticles, Poultry meat

## INTRODUCTION

With a low cost of production and a large range of recipes, chicken meat is an excellent alternative to red meat, since it is high in protein, vital amino acids, fatty acids, and vitamins (Connolly and Campbell, 2023). Although customers frequently like chicken meat, its bacteriological quality may provide a hazardous danger to human health. For this reason, guaranteeing the microbiological safety of chicken meat products is crucial in light of rising production and consumption (Tahir *et al.*, 2023).

Indeed, the environment of the slaughterhouse, the bacteria from the intestinal microbiota, and the used equipment can

Corresponding author: MOHAMED NABIL
*E-mail address:* mhmdvet2010@gmail.com
*Present address:* Department of Food Hygiene, Animal Health Research Institute (AHRI) – Benha Lab., Agriculture Research Center (ARC), Egypt.

contaminate carcasses throughout the slaughtering process. This contamination may be as high as serious food pathogens like *Salmonella* and *Campylobacter spp.*, which are the two main pathogens that cause human food poisoning following consumption of contaminated foods (Rouger *et al.*, 2017).

*Campylobacter species*, *C. jejuni*, *C. coli* and *C. fetus* particularly, are negative-Gram stain, helical-shaped microaerophilic bacteria that may be found as dormant pathogens in the intestinal tract of chicken; and usually incriminated in campylobacteriosis in humans following consumption of undercooked chicken meats (Costa and Iraola, 2019).

Campylobacter is highly prevalent in chicken meat worldwide, including broilers, layers, turkeys, ducks, and geese. It is a commensal bacterium that establishes persistent infections with colonization levels up to $10^{10}$ CFU/g gram of microbiota in broilers (El-Saadony *et al.*, 2023).

One of the primary causes of campylobacteriosis linked to *C. jejuni* and its close genus *C. coli* is the consumption of undercooked or cross-contaminated chicken meat (CDC, 2024). About 10% of the world's population, including 200 million children, suffer from diarrheal diseases each year due to *C. jejuni* gastroenteritis, which has been reported as the most common gastrointestinal bacterial pathogen in humans in the EU since 2005. The symptoms of this illness range from mild, non-inflammatory diarrhea to severe abdominal cramps and bloody diarrhea that necessitates hospitalization and antimicrobial treatment (EFSA, 2016 and WHO, 2020).

Consumers' interest in green-preservation of meat products has motivated researchers to evaluate the effectiveness and applicability of many natural compounds with antioxidant and antimicrobial purposes (Roila *et al.*, 2022). Consequently, there has been a noticeable surge in the search for natural additives, particularly those derived from plants, in recent years, suggesting that the use of natural antimicrobial activity may be helpful for preserving meat quality, increasing shelf life, and improving human health (Petcu *et al.*, 2023).

Green tea, *Camellia sinensis*, leaves extract is an important choice as a safe plant-meat-additive because of its potent antioxidant, deodorant and antimicrobial activities revealing longer shelf-life of foods, enhance quality and provide safer meat product (Zhao *et al.*, 2022).

The antimicrobial effect of green tea extract was repeatedly recorded in several studies against wide range of foodborne pathogens even by itself (Alghamdi, 2023), or in combination with other nanoparticles (NPs) of copper (Anna Thomas *et al.*, 2022), chitosan (Sathiyabama *et al.*, 2024), and silver (Safa and Koohestani, 2024); even, it gave more potent antibacterial effect than silver NPs that give a chance for avoid mineral NPs side effects (Raza *et al.*, 2023); that may be attributed to its significant contents of flavonoids, alkaloids, phenolic compounds and tannins (Pérez-Burillo *et al.*, 2021).

The use of nanoparticles in the processing, preservation, and preparation of meat products is becoming more popular as a clever tool for a safer, more economical, and sustainable food chain. The shelf-life, nutritional value, and sensory qualities of meat products can all be enhanced by using nanoparticles to support the preservation process. Consequently, nanoparticles can effectively help guarantee product quality and safety while cutting expenses and waste (Ulloa-Saavedra *et al.*, 2022).

Therefore, the current study was planned to determine the prevalence of *Campylobacter spp.* in chicken meat samples accompanied by experimental assessment of the antibacterial effect of green tea leaves extract nanoparticles against chicken-borne campylobacter in chicken fillet samples during refrigeration.

## MATERIALS AND METHODS

### 1. Bacteriological examination

### 1.1. Collection of samples

Fifty raw and fresh varied samples of chicken meat were collected from random butcher shops and markets in Qalubiya government. Samples were transferred to the lab in a hygienic manner, followed by the bacteriological examination as soon as possible.

### 1.2. Isolation and identification of Campylobacter spp.

**ISO 10272-1 (2017a)** guidelines were followed in order to detect *Campylobacter spp.* in 25g of each sample using the standard technique. Preston selective broth (Oxoid) containing 5% sterile lysed horse blood was used to enrich each sample. The samples were then incubated for twenty-four hours at forty-two Celsius degrees in a microaerobic environment. Modified charcoal-cefoperazone-deoxycholate agar (mCCDA) supplemented with sterile lysed horse blood was used to streak enriched cultures. Microaerobic conditions were used to incubate streaked cultures for forty-eight hours at forty-two Celsius degrees. After being chosen and purified, suspected campylobacter colonies were identified by morphology and biochemistry. Hydrolysis of hippurate was used to differentiate between *C. jejuni* and *C. coli* according to ISO 10272-1 (2017a).

### 1.3. Molecular examination of some virulence factors of *C. jejuni* isolates

### 1.3.1. DNA Extraction

DNA preparation from bacterial culture was performed, according to Shah *et al.* (2009), following the instruction of QIAamp DNA mini kit instructions (Catalogue no.51304).

### 1.3.2. Oligonucleotide primers used in PCR

The examined target *cdtA* and *cdtB*gene sequences of *C. jejuni* are demonstrated in Table (1), respectively.

**Table (1):** Primers sequences, target genes, amplicon sizes and cycling conditions.

| Target gene | | Primers sequences (3' - 5') | Amplified segment | Reference |
|---|---|---|---|---|
| *cdtA* | F | GGAAATTGGATTTGGGGCTATACT | 165 bp | Bang *et al.* (2003) |
| | R | ATCAACAAGGATAATGGACAAT | | |
| *cdtB* | F | CAGAAAGCAAATGGAGTGTT | 220 bp | Nahar and Bin Rashid (2018) |
| | R | AGCTAAAAGCGGTGGAGTAT | | |

**1.3.3. Agarose gel electrophoreses** was performed according to **Sambrook *et al.* (1989).**

### 2. Experimental controlling of *C. jejuni* in chicken fillet

### 2.2. Preparation of green tea leaves extract (GTE) loaded nanoparticles

Ready to use GTE nanoparticles was obtained from the nanotechnology unit, Animal Health Research Institute; where the production of nano-sized material was performed and characterized using Microtrac® nano-sizer.

### 2.1. Evaluation of the nano-material cytotoxicity

Cell viability was assessed by MTT assay with different concentrations (0, 6.25, 12.5, 25.0, 50 and 100 µg/ml), according to Mosmann (1983), using Vero cell (Green monkey cell line) obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Briefly, 104-106 cells were plated in 200 µl PBS in 96 well flat-bottomed plate, followed by addition of 20 ml of MTT solution and mixed well. After incubation at 37°C for 4 h. In a dark place, 200 ml of acidic isopropanol was added and mixed well, followed by incubation at 37°C for 1h.

after which, plates were read in ELISA Reader at 570 nm optical density.

### 2.2. Experimental design

Total of 525 g of chicken meat fillet was obtained from Benha city's local markets, Qalubiya governorate, followed by bacteriological examination according to ISO 10272-1 (2017a) to ensure it is free of campylobacter contamination.

Chicken fillet samples were inoculated experimentally with *C. jujeni* (nearly $10^3$ CFU/g). Then, it was divided into three main groups (175 g / group). Inoculated samples were left in the refrigerator for about fifteen minutes for acclimatization of microorganisms, before addition of GTE nanoparticles. G1 represented control positive group treated only with normal saline; G2 represented treated group after addition of 250 ppm GTE nanoparticles; G3 represented treated group after addition of 500 ppm GTE nanoparticles.

Sensory quality and campylobacter counting were performed every 48h of refrigeration until apparently spoiled. Results of triplicate trials were recorded and evaluated.

### 2.3. Sensory evaluation

Following Mörlein (2019), samples were physically inspected, and the average color, odor, and texture ratings were recorded as total sensory scores. Ratings ranged from 1 to 5, with 5 denoting excellent and ≤1 denoting the worst.

### 2.4. Bacterial counting

*Campylobacter jejuni* counting was performed following ISO 10272-2 (2017b) guidelines. All experiments were conducted in triplicate.

### 3. Statistical analysis

SPSS version 20 was employed for data analysis. ANOVA analysis and the Duncan posthoc value were used to assess the significance of the differences in the mean values of the groups being studied; when the control group was spoiled, an independent T-test was employed. $P < 0.05$ was considered a substantial significance level.

## RESULTS

### 1. Bacteriological findings

Out of fifty chicken meat samples examined, *Campylobacter species* was isolated from nineteen samples with an incidence of 38%. *C. jejuni* recorded higher prevalence with 30%, while *C. coli* was detected in 8% of the samples examined (Fig. 1).

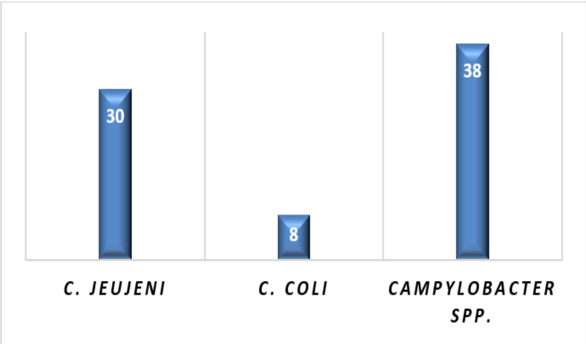


**Fig. (1):** Prevalence of *Campylobacter species* in the examined chicken meat samples.

Molecular detection of *cdtA* and *cdtB* genes in *C. jejuni* isolates, as virulence factors, revealed positive detection of *cdtA* gene in all examined isolates (100%). Whereas *cdtB* was detected in only two isolates with prevalence of 40.0%.

### 2. Experimental investigation of the effect of green tea extract NPs

#### 2.1. Size characterization of nanomaterial

Green tea leaves nanoparticle size was determined by Microtrac® size analyzer, and the results revealed that the particle size was 6.31 nm (Fig. 3).

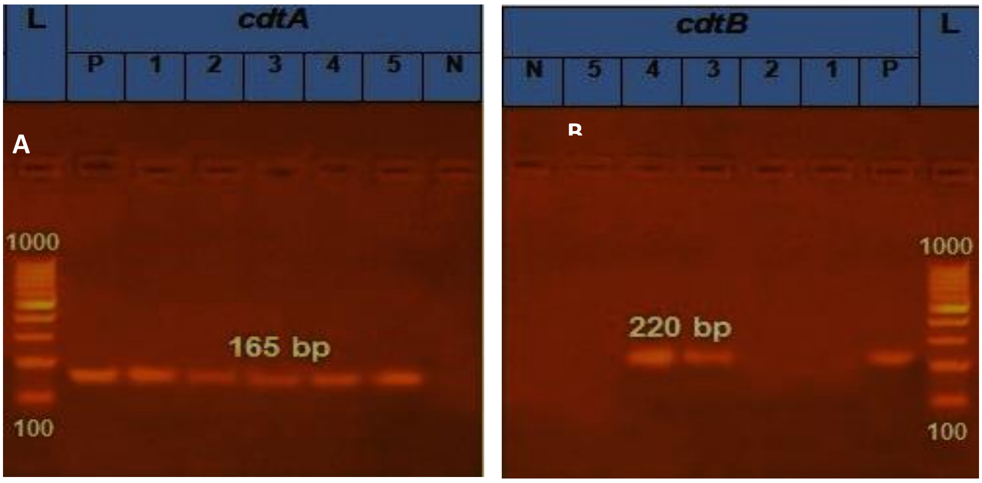


**Fig. (2):** Amplification of the *cdtA* (A) (165 bp) and *cdtB* (B) (220 bp) genes in isolates of *C. jejuni* using agarose gel electrophoresis. 100 bps DNA StepLadder, positive control (*C. jejuni*), negative control (*S. aureus*) were inoculated in Lane (L), Lane (P), and Lane (N), respectively. *cdtA* gene amplified at 165 bp in lanes 1 to 5. While, *cdtB*gene was only amplified in samples of lane 3 and 4, whereas isolates of lanes 1, 2 and 5 were negative for *cdtB* gene.

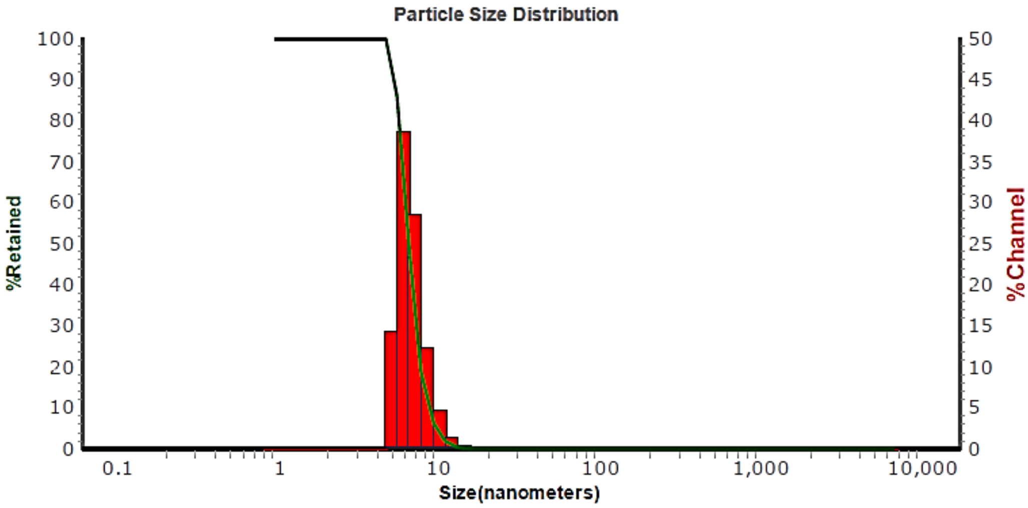


**Fig. (3):** Particle size distribution of GTE

**2.2. Results of cytotoxicity assay**

Regarding the safety assessment of the used nanomaterial, (Table 2 and Fig. 4) revealed that IC50 was 192.2, where the cell viability was 60.67% when the maximum concentration (100%) was used.

**Table (2):** Cell viability and IC50 of the used GTE nanomaterial.

| Concentration of GTE | Mean ± SD | Cell viability % | IC50 % |
|---|---|---|---|
| **0** | 0.297 ± 0.011 | 100 | **192.2** |
| **6.25** | 0.280 ± 0.004 | 94.60 | |
| **12.5** | 0.253 ± 0.006 | 85.27 | |
| **25.0** | 0.239 ± 0.008 | 80.67 | |
| **50.0** | 0.223 ± 0.002 | 75.28 | |
| **100.0** | 0.180 ± 0.006 | 60.67 | |

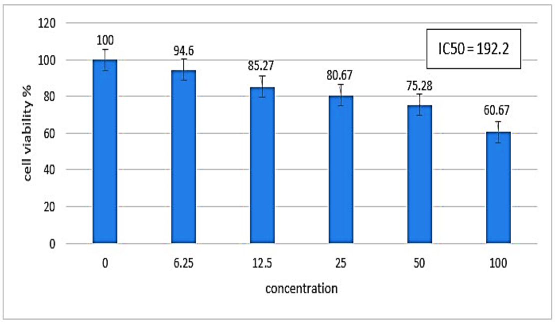


**Fig. (4):** Cell viability in relation to the concentration of the used nanomaterial.

### 3. Overall organoleptic acceptability of the samples treated

Regarding the organoleptic acceptability scores of the examined chicken fillet, the control group showed rapid deterioration and became apparently spoiled after the 6th day of refrigeration. Whereas GTE treated groups revealed longer time with higher acceptability scores along the experiment time. Physical enhancement effect of GTE proved to be concentration dependent, where the treatwhereasen fillet with 500 ppm GTE preserved its acceptability up to 10 days of storage (Fig. 5). According to this figure, if the final quality score is 2, the sample's quality is marginally acceptable. If this score is less than 2, the sample is unacceptable. If this score is less than 1, the sample is apparently spoiled.

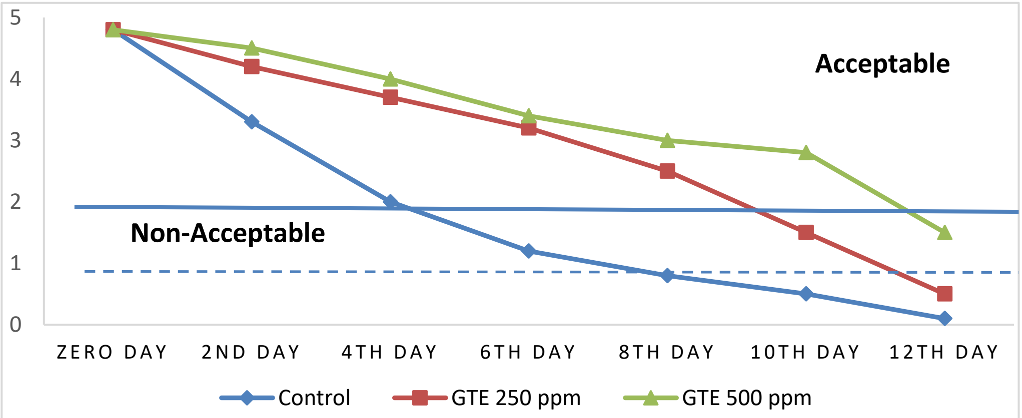


**Fig. (5):** Sensory profile of the examined chicken groups in cold storage (4±1$^{O}$C).

### 4. Anti-campylobacter effect of the used GTE nanomaterial

Regarding the anti-campylobacter effect of GTE in the inoculated samples (Tables, 3 and 4, and Fig. 6), significant reductions in C. jejuni count were recorded in the treated samples, where higher reduction percentage was recorded in higher GTE concentration treated samples. Campylobacter counts showed significant decline from 3.2 log CFU/g (zero day) to 1.8 and 1.2 log CFU/g (12th day of storage), with reduction percent of 43.8 and 62.5%, respectively. On the other hand, control untreated group, showed multiplication in campylobacter count up to 4.5 log CFU/g at the 12th day of storage.

**Table (3)**: Mean counts±SD of *C. jejuni* in relation to storage time and nanomaterial concentration

| | Concentration of the nanomaterial | | |
|---|---|---|---|
| **Time** | Control | 250 ppm | 500 ppm |
| **Zero day** | 3.2±0.06Ea | 3.2±0.06Aa | 3.2±0.06Aa |
| **3rd day** | 3.5±0.1Da | 2.8±0.1Bb | 2.6±0.1Bc |
| **6th day** | 3.8±0.1Ca | 2.5±0.1Cb | 2.1±0.1Cc |
| **9th day** | 4.2±0.2Ba | 2.0±0.1Db | 1.5±0.1Dc |
| **12th day** | 4.5±0.1Aa | 1.8±0.1Eb | 1.2±0.2Ec |

AB. Capital superscript letters within the same column indicate a significant difference (P≤0.05)
ab. Small superscript letters within the same row indicate significant difference (P≤0.05)

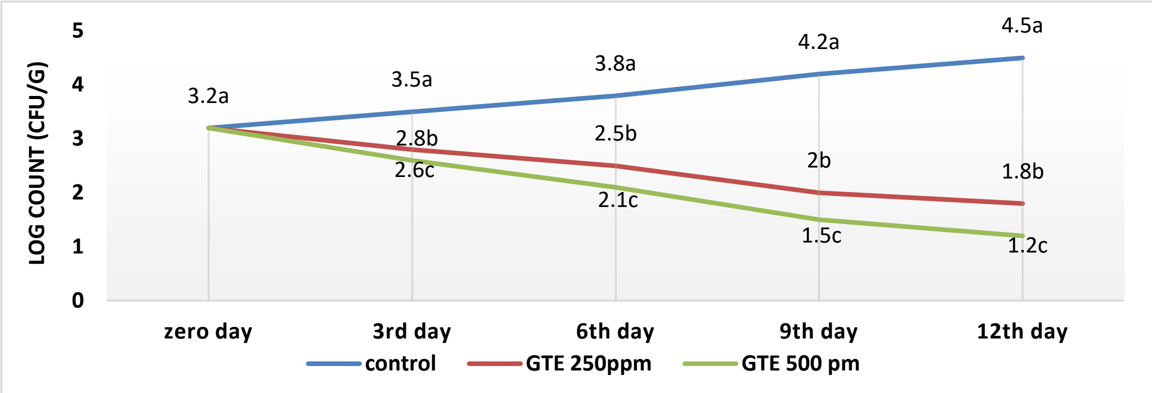


**Fig. (6):** Effect of different concentrations of GTE on *Campylobacter jejuni* mean counts (log CFU/g) in control and treated samples. *Means carrying different superscript small letters are significantly different (P < 0.05).

**Table (4):** Reduction % of *C. jejuni* counts in GTE treated chicken fillet

| | GTE 250 ppm | GTE 500 ppm |
|---|---|---|
| **Zero day** | -- | -- |
| **3rd day** | 12.5 | 18.8 |
| **6th day** | 21.9 | 37.5 |
| **9th day** | 37.5 | 53.1 |
| **12th day** | 43.8 | 62.5 |

Reduction % was calculated in relation to control reading at zero day (3.2 log CFU/g)

## DISCUSSION

Chicken meat is a significant promoting food item related to human health because of its high nutritional profile, affordability and easy digestion (Jenkins *et al*., 2016). On the other hand, the public health significance of *Campylobacter species*, particularly *C. jejuni*, in chicken meat is a leading cause of foodborne illness globally. This bacterium is commonly found in the intestines of poultry, making chicken a primary reservoir for transmission to humans. Consumption of undercooked or contaminated chicken is the major risk factor for campylobacteriosis, which presents symptoms such as diarrhea, fever, and abdominal cramps (Al Hakeem *et al*., 2022).

Regarding the present recorded results of the prevalence of *Campylobacter spp.* in the examined samples (Fig. 1), it was detected in 38.0% with superiority of *C. jejuni* over *C. coli*. The obtained results came in line with those of Mohamed (2019), who found that *Campylobacter spp.* was detected in 32.0% of the examined chicken meat samples. Whereas lower prevalence (25.4%) was recorded (Poudel *et al*. 2022). Another study found an exceptionally high prevalence of 73.86% in fresh chicken meat. Whereas *C. jejuni* and *C. coli* were detected in 53.53% and 15.35% of the samples, respectively (Mikulić *et al*., 2016).

*Campylobacter jejuni* is a significant food-borne pathogen known for its virulence factors, particularly the cytolethal distending toxin (CDT), which is composed of *cdtA*, *cdtB*, and *cdtC* subunits that play crucial roles in the bacterium's pathogenicity (Le *et al*., 2024).

The action of CDT is associated with significant cellular damage and contributes to inflammatory responses within the host, that may enhance the invasiveness of *C. jejuni*, allowing it to persist in host tissues and evade immune responses (Lopes *et al*., 2021).

The *cdtA* and *cdtB* genes are crucial for the synthesis of their respective toxin subunits. Studies have shown that *C. jejuni* strains lacking these genes exhibit reduced virulence compared to pathogenic strains, highlighting their importance in pathogenesis. Studies indicated that these genes are commonly found in *C. jejuni* isolates from both poultry and human sources, reinforcing their role as key virulence determinants (Sierra-Arguello *et al.*, 2021).

Referring to the current recorded results (Fig., 2A and 2B); it came in line with the recorded results by de Carvalho *et al.* (2014), who found cdtA of C. jejuni strains in prevalence of 83.3%, and Salem *et al.* (2019), who found cdtA and *cdtB* genes in *C. jejuni* isolates in prevalence of 75.0 and 70.0%, respectively; therefore, the positive detection of *cdt* genes indicating the virulence and pathogenicity of *campylobacter spp.*

Several health measures have been recommended for mitigation of the risks associated with *C. jejuni* contamination in the food sector, that include implementing strict biosecurity protocols on poultry farms, improving processing hygiene, and promoting safe cooking practices among consumers (Al Hakeem *et al.*, 2022). On the same line, researchers are welling to substitute the chemically synthetic food additives with natural potent preservatives with antimicrobial function in compliance with the consumer's preference (Zhong *et al.*, 2018).
Nanotechnology is revolutionizing food preservation by enhancing safety, extending shelf life, and improving the overall quality of food products. This innovative technology utilizes nanoscale materials and processes to create more effective preservation methods than the raw material, compared to traditional techniques (Biswas *et al.*, 2022).

The use of green tea leaves extract (GTE), particularly its active components like catechins, has shown promising antimicrobial and preservative effects in meat products. These properties are primarily attributed to the extract's ability to minimize the growth of various pathogenic bacteria, making it a viable alternative to synthetic preservatives (Dai *et al.*, 2022).

Nanotechnology presents promising advancements in food preservation. However, it faces several limitations, particularly regarding safety and efficacy. One significant concern is the potential migration of nanoparticles into food products, which raises questions about their toxicity to humans and the environment, necessitating stringent regulatory measures that vary by region (Pattnaik *et al.*, 2024).

Referring to the recorded results of cytotoxicity MTT assay in the present study, the used GTE nanomaterial revealed a high safety level that came in line with the recorded statements of EFSA (2018) and Health Canada safety assessment (2023) which concluded that extracts from traditional green tea leaves are generally safe when consumed at typical intake levels.

Referring to the present study, treatment of chicken fillet with GTE nanomaterial revealed significant enhancement in the sensory acceptability, with notable elongation in the organoleptic acceptability of the treated samples compared with the untreated control samples; which came in line with the recorded results of Salem *et al.* (2016), who reported that the incorporation of green tea extract into meat products revealed significant improvement in the acceptability criteria alongside its antimicrobial and antioxidant properties. Moreover, Ucak *et al.* (2011) and Pateiro *et al.* (2015) recorded that green tea extract lowers total color difference throughout twenty-four weeks of refrigerated stored the treated meat samples.

The recorded enhanced sensory quality of the GTE treated chicken meat samples may be attributed to its content of polyphenols, particularly catechins, which can influence the flavor profile of meat products. In addition, green tea extract has been shown to improve color stability in meat products because of its potential to reduce lipid oxidation, which helps maintain the desirable color of meat during storage (Nain *et al.*, 2022). Furthermore, GTE have demonstrated significant antibacterial activity against *C. jejuni*, which contributes to improved sensory characteristics by reducing spoilage and off-flavors associated with microbial growth, leading to less microbial spoilage and better preservation of sensory qualities.

In addition, the use of GTE NPs presented a promising approach to control *C. jejuni* in chicken meat, addressing a significant public health concern through its antibacterial properties, as it is rich in polyphenols, particularly catechins, which exhibit strong antibacterial through interference with bacterial cell membranes and metabolic processes, leading to cell death activity against various pathogens, including *C. jejuni*.

Figure (4) and Table (2) showed significant reductions in the *C. jejuni* counts along the experiment time; where it was concentration-time dependent; whereas, higher concentration of GTE revealed higher reductions.

The obtained results came in line with the recorded results of Diker *et al.* (1991) and Liu *et al.* (2022) who determined the bactericidal effect of GTE on different foodborne pathogens including *C. jejuni*, and revealed that significant decline in the bacterial count within 4h from incubation in vitro; Kurekci *et al.* (2013) who recorded significant inhibitory effect of 1.0% green tea oil on *C. jejuni* growth; Castillo *et al.* (2015) who found that the extracted tea polyphenols prohibited the movement and biofilm formation of *C. jejuni;* Zhang *et al.* (2021) who recorded a significant stable antibacterial effect of green tea polyphenols on wide range of foodborne pathogens including *C. jejuni*; and Alghamdi (2023) who recorded potent bactericidal effect of green tea different types of extracts against both Gram-positive and Gram-negative foodborne pathogens by the concentration of 20.0%.

Despite the agreement of all previous records on the potent antibacterial effect of GTE, particularly against *C. jejuni*, variations were observed in the potentiality of GTE to reduce the bacterial counts, that may be attributed to the dose of GTE, method of extraction and the matrix of investigation, either in vivo or in vitro. Besides that, the current used GTE NPs revealed higher reduction ability against the bacterial counts of *C. jejuni* that may be attributed to the maximized spread and invasiveness of the nano-size materials, compared with raw substances (Patra *et al.*, 2018).

## CONCLUSION

After all, the present study revealed that chicken meat can be categorized as one of the possible sources of campylobacter food poisoning. *Campylobacter jejuni* was detected in higher rates, compared with *C. coli*. The presence of such cytolethal distending toxin (cdt)-positive campylobacter strains indicated potent, virulent and toxigenic isolates. Moreover, application of GTE NPs showed significant improving effects on the chicken fillet physical characters, with potential potent anti-campylobacter effects during refrigeration. To our knowledge, this is the first study in Egypt that investigate the ability of nano-green tea leaf extract to inhibit the growth of *C. jejuni* in chicken fillet.

## REFERENCES

*Al Hakeem, W.G.; Fathima, S.; Shanmugasundaram, R. and Selvaraj, R.K. (2022): Campylobacter jejuni* in poultry: Pathogenesis and control strategies. Microorganisms, 10(11): 2134.

*Alghamdi, A.I. (2023):* Antibacterial activity of green tea leaves extracts against specific bacterial strains. J. King Saud Univ. - Science, 35(5): 102650.

*Anna Thomas, A.; Varghese, R.M. and Rajeshkumar, S. (2022):* Antimicrobial effects of copper nanoparticles with green tea and neem formulation. Bioinformation, 18(3): 284-288.

*Bang, D.D.; Nielsen, E.M.; Scheutz, F.; Pedersen, K., Handberg, K. and Madsen, M. (2003):* PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. J. Appl. Microbiol., 94, 1003–1014.

*Biswas, R.; Alam, M.; Sarkar, A.; Haque, M.I.; Hasan, M.M. and Hoque, M. (2022):* Application of nanotechnology in food: processing, preservation, packaging and safety assessment. Heliyon, 8(11): 11795.

*Castillo, S.; Heredia, N. and García, S. (2015):* 2(5H)-furanone, epigallocatechin gallate, and a citric-based disinfectant disturb quorum-sensing activity and reduce motility and biofilm formation of *Campylobacter jejuni.* Folia Microbiol., 60, 89-95.

*CDC "Center for Disease and Control" (2024):* About *Campylobacter* infection. Available on: https://www.cdc.gov/campylobacter/about/index.html. Visited at July 2024.

*Connolly, G. and Campbell, W.W. (2023):* Poultry consumption and human cardiometabolic health-related outcomes: A narrative review. Nutrients, 15(16): 3550.

*Costa, D. and Iraola, G. (2019):* Pathogenomics of emerging Campylobacter species . Clin. Microbiol. Rev., 32, 72-118.

*Dai, J.; Sameen, D.; Zeng, Y.; Li, S.; Qin, W. and Liu, Y. (2022):* An overview of tea polyphenols as bioactive agents for food packaging applications. LWT, 167, 113845.

*de Carvalho, A.F.; da Silva, D.M.; Azevedo, S.S.; Piatti, R.M.; Genovez, M.E. and Scarcelli, E. (2014):* Detection of CDT toxin genes in Campylobacter spp. strains isolated from broiler carcasses and vegetables in São Paulo, Brazil. Braz. J. Microbiol., 44(3): 693-699.

*Diker, K.S.; Akan, M.; Hascelik, G. and Yurdako, M. (1991):* The bactericidal activity of tea against *Campylobacter jejuni* and *Campylobacter coli.* Lett. Appl. Microbiol., 12, 34-35.

*EFSA (2016):* The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA. J., 14, 4634.

*EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Younes, M.; Aggett, P.; Aguilar, F.; Crebelli, R.; Dusemund, B.; Filipič, M.; Frutos, M.J.; Galtier, P.; Gott, D.; Gundert-Remy, U.; Lambré, C.; Leblanc, J.C.; Lillegaard, I.T.; Moldeus, P.; Mortensen, A.; Oskarsson, A.; Stankovic, I.; Waalkens-Berendsen, I.; Woutersen, R.A. and Wright, M. (2018):* Scientific opinion on the safety of green tea catechins. EFSA J., 16(4), 5239.

*El-Saadony, M.T.; Saad, A.M.; Yang, T.; Salem, H.M.; Korma, S.A.; Ahmed, A.E.; Mosa, W.F.A.; Abd El-Mageed, T.A.; Selim, S.; Al Jaouni, S.K.; Zaghloul, R.A.; Abd El-Hack, M.E.; El-Tarabily, K.A. and Ibrahim, S.A. (2023):* Avian campylobacteriosis, prevalence, sources, hazards, antibiotic resistance, poultry meat contamination, and control measures:

A comprehensive review. Poult. Sci., 102(9): 102786.

*Health Canada safety assessment (2023):* Summary of health Canada's safety assessment of green tea extract for use as a supplemental ingredient. [Online source] retrieved on Jan. 2025. Available on https://www.canada.ca/en/health-canada/services/food-nutrition/public-involvement-partnerships/notice-modification-list-permitted-supplemental-ingredients-permit-use-green-tea-extract-supplemental-ingredient-foods/document.html

*ISO "International Organization for Standardization" (2017a):* No. 10272-1. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. Part 1: Detection method.

*ISO "International Organization for Standardization" (2017b):* No. 10272-2. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. Part 2: Colony count technique.

*Jenkins, T.A.; Nguyen, J.C.; Polglaze, K.E. and Bertrand, P.P. (2016):* Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. Nutrients, 8(1): 56.

*Kurekci, C.; Padmanabha, J.; Bishop-Hurley, S.L.; Hassan, E.; Al Jassim, R.A. and McSweeney, C.S. (2013):* Antimicrobial activity of essential oils and five terpenoid compounds against *Campylobacter jejuni* in pure and mixed culture experiments. Int. J. Food Microbiol., 166(3): 450-457.

*Le, L.; Elgamoudi, B.; Colon, N.; Cramond, A.; Poly, F.; Ying, L.; Korolik, V. and Ferrero, R.L. (2024): Campylobacter jejuni* extracellular vesicles harboring cytolethal distending toxin bind host cell glycans and induce cell cycle arrest in host cells. Microbiol. Spectr., 12(3): 323223.

*Liu, S.; Zhang, Q.; Li, H.; Qiu, Z. and Yu, Y. (2022):* Comparative assessment of the antibacterial efficacies and mechanisms of different tea extracts. Foods, 11(4), 620.

*Lopes, G.; Ramires, T.; Kleinubing, N.; Scheik, L.; Fiorentini, A. and da Silva, D. (2021):* Virulence factors of foodborne pathogen *Campylobacter jejuni*. Microb. Path., 161, 105265.

*Mikulić, M.; Humski, A.; Njari, B.; Ostović, M.; Duvnjak, S. and Cvetnić, Ž. (2016):* Prevalence of thermotolerant Campylobacter spp. in chicken meat in Croatia and multilocus sequence typing of a small subset of *Campylobacter jejuni* and *Campylobacter coli* isolates. Food Technol. Biotechnol., 54(4): 475-481.

*Mosmann, T. (1983):* Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65, 55-63.

*Mohamed, K. (2019):* Prevalence of Campylobacter spp. and its pathogenic genes in poultry meat, human and environment in Aswan, Upper Egypt. Assiut Vet. Med. J., 65, 151-158.

*Mörlein, D. (2019):* Sensory evaluation of meat and meat products: Fundamentals and applications. The 60th International Meat Industry Conference MEATCON2019. IOP Conf. Series: Earth and Environmental Science. doi:10.1088/1755-1315/333/1/012007

*Nahar, N. and Bin Rashid, R. (2018):* Genotypic analysis of the virulence and antibiotic resistance genes in Campylobacter species in silico. J. Bioanal. Biomed., 10, 1-15.

*Nain, C.W.; Mignolet, E.; Herent, M.F.; Quetin-Leclercq, J.; Debier, C.; Page, M.M. and Larondelle, Y. (2022):* The catechins profile of green tea extracts affects the antioxidant activity and degradation of catechins in DHA-rich oil. Antioxidants, 11(9): 1844.

*Pateiro, M.; Lorenzo, J.M.; Vazquez, J.A. and Franco, D. (2015):* Oxidation stability of pig liver pate with increasing levels of natural antioxidants (Grape and Tea). Antioxidants, 4, 102-123.

*Patra, J.K.; Das, G. and Fraceto, L.F. (2018):* Nano based drug delivery systems: recent developments and future prospects. J. Nanobiotechnol, 16, 71.

*Pattnaik, R.; Panda, S.K.; Biswas, S.; De, S.; Satahrada, S. and Kumar, S. (2024):* Prospects and challenges of nanomaterials in sustainable food preservation and packaging: a review. Discover nano, 19(1), 178.

*Pérez-Burillo, S.; Navajas-Porras, B.; López-Maldonado, A.; Hinojosa Nogueira, D.; Pastoriza, S. and Rufián Henares, J.Á. (2021):* Green tea and its relation to human gut microbiome. Molecules, 26(13): 3907.

*Petcu, C.D.; Mihai, O.D.; Tăpăloagă, D.; Gheorghe-Irimia, R.A.; Pogurschi, E.N.; Militaru, M.; Borda, C. and Ghimpețeanu, O.M. (2023):* Effects of plant-based antioxidants in animal diets and meat products: A review. Foods, 12(6): 1334.

*Poudel, S.; Li, T.; Chen, S.; Zhang, X.; Cheng, W.H.; Sukumaran, A.T.; Kiess, A.S. and Zhang, L. (2022):* Prevalence, antimicrobial resistance, and molecular characterization of Campylobacter isolated from broilers and broiler meat raised without antibiotics. Microbiol. Spectr., 10(3): 25122.

*Raza, S.; Wdowiak, M.; Grotek, M.; Adamkiewicz, W.; Nikiforow, K.; Mentea, P. and Paczesny, J. (2023):* Enhancing the antimicrobial activity of silver nanoparticles against ESKAPE bacteria and emerging fungal pathogens by using tea extracts. Nanoscale Adv., 5, 5786.

*Roila, R.; Sordini, B.; Esposto, S.; Ranucci, D.; Primavilla, S.; Valiani, A.; Taticchi, A.; Branciari, R. and Servili, M. (2022):* Effect of the application of a green preservative strategy on minced meat products: Antimicrobial efficacy of olive mill waste water polyphenolic extract in improving beef burger shelf-life. Foods, 2022, 11.

*Rouger, A.; Tresse, O. and Zagorec, M. (2017):* Bacterial contaminants of poultry meat: Sources, species, and dynamics. Microorganisms, 5(3): 50.

*Safa, M. and Koohestani, H. (2024):* Green synthesis of silver nanoparticles with green tea extract from silver recycling of radiographic films. Engineering, 21, 101808.

*Salem, A.; Abou El Roos, N. and Nassar, Y. (2019):* Molecular and conventional methods for detection of Campylobacter spp. in retail poultry cuts, offal and chicken products. Benha J. Vet. Med., 36, 71-76.

*Salem, A.; Khater, D. and El Barody, S. (2016):* Effect of rosemary and green tea extracts on overall quality and shelf-life of meat during storage. Benha Vet. Med. J., 31, 17-23.

*Sambrook, J.; Fritscgh, E. and Mentiates, A. (1989):* Molecular cloning. A laboratory manual. Vol. 2, Cold spring Harbor Laboratotry press, New York.

*Sathiyabama, M.; Boomija, R.V. and Muthukumar, S. (2024):* Green synthesis of chitosan nanoparticles using tea extract and its antimicrobial activity against economically important phytopathogens of rice. Sci. Rep., 14, 7381.

*Shah, D.; Shringi, S.; Besser, T. and Call, D. (2009):* Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In: Liu, D. (Ed). Taylor & Francis group, Florida, USA, Pp. 369389.

*Sierra-Arguello, Y.M.; Perdoncini, G.; Rodrigues, L.B.; Dos Santos, R.L.;*

*Apellanis Borges, K.; Quedi Furian, T.; Pippi Salle, C.T.; de Souza Moraes, H.L.; Pereira Gomes, M.J. and Pinheiro do Nascimento, V. (2021):* Identification of pathogenic genes in *Campylobacter jejuni* isolated from broiler carcasses and broiler slaughterhouses. Sci. Rep., 11(1): 4588.

*Tahir, M.; Park, S.; Anwar, I.; Bilal, R.; Hussain, K.; Muneeb, M.; Rehman, A.; Abbas, A. and Ghumman, N. (2023):* Foodborne pathogens in poultry: A public health concern. In: Khan A, Rasheed M and Abbas RZ (eds), Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan, 1st Ed., pp. 447-458.

*Ucak, I.; Ozogul, Y. and Durmus, M. (2011):* The effects of rosemary extract combination with vacuum packing on the quality changes of Atlantic mackerel fish burgers. Int. J. Food Sci. Technol., 46, 1157-1163.

*Ulloa-Saavedra, A.; García-Betanzos, C.; Zambrano-Zaragoza, M.; Quintanar-Guerrero, D.; Mendoza-Elvira, S. and Velasco-Bejarano, B. (2022):* Recent developments and applications of nanosystems in the preservation of meat and meat products. Foods, 11, 2150.

*WHO "World Health Organization" (2020):* Campylobacter. WHO Fact Sheets. Available on https://www.who.int/news-room/fact-sheets/detail/campylobacter. Visited in July 2024.

*Zhang, Q.; Zhang, J.; Zhang, J.; Xu, D.; Li, Y.; Liu, Y.; Zhang, X.; Zhang, R.; Wu, Z. and Weng, P. (2021):* Antimicrobial effect of tea polyphenols against foodborne pathogens: A review. J. Food Prot., 84, 1801-1808.

*Zhao, T.; Li, C.; Wang, S. and Song, X. (2022):* Green tea (*Camellia sinensis*): A review of its phytochemistry, pharmacology, and toxicology. Molecules, 27(12): 3909.

*Zhong, Y.; Wu, L.; Chen, X.; Huang, Z. and Hu, W. (2018):* Effects of food-additive-information on consumers' willingness to accept food with additives. Int. J. Environ. Res. Public Health, 15(11): 2394.

# نظرة عامة على التأثير المضاد للبكتريا لجزيئات مستخلص الشاي الأخضر النانوية على بكتيريا الكامبيلوباكتر المعزولة من لحم الدجاج

***محمد نبيل ، ايناس على ، نسمة جمال ، رباب رواش ، علا فتحى ، لمياء عطيه ، رحاب جعفر و ناريمان عبد الهادى***

Email address: mhmdvet2010@gmail.com Assiut University web-site: www.aun.edu.eg

تعد عائلة بكتريا الكامبيلوباكتر، وخاصةً سلالات الكامبيلوباكتر جيجوناي وكامبيلوباكتر كولاي، من مسببات التسمم الغذائي والتي توجد عادةً في لحوم الدجاج. لذلك، هدفت الدراسة الحالية إلى تحديد مدى تواجد أنواع الكامبيلوباكتر في عدد خمسين عينة عشوائية من عينات لحوم الدجاج النيئة، والتي تم جمعها من محلات بيع الدواجن بمحافظة القليوبية، جمهورية مصر العربية. أظهرت النتائج إيجابية عزل بكتريا الكامبيلوباكتر في اجمالي ٣٨,٠٪ من العينات التي تم فحصها؛ حيث بلغت نسبة تواجد الكامبيلوباكتر جيجوناي و كامبيلوباكتر كولاي ٣٠,٠ و ٨,٠٪، على التوالي. من ناحية أخرى، تم اختبار ضراوة معزولات الكامبيلوباكتر جيجوناي جزيئيًا من خلال الكشف عن جينات *cdtA* و *cdtB* والتي وجدت في ١٠٠٪ و ٤٠,٠٪ من المعزولات المفحوصة، على التوالي. ولأهمية إيجاد إضافات طبيعية يمكنها تقليل انتشار بكتريا الكامبيلوباكتر في لحوم الدجاج، تم استخدام مستخلص أوراق الشاي الاخضر النانوية وقياس تأثيرها المضاد لبكتريا الكامبيلوباكتر جيجوناي بتركيز ٢٥٠ و ٥٠٠ جزء في المليون في فيليه الدجاج المحقونة تجريبياً أثناء الحفظ بالتبريد. أظهرت النتائج قدرة مستخلص الشاي الاخضر على تحسين درجات القبول الحسي في العينات المعالجة مع إطالة فترة صلاحية عينات الدجاج ظاهرياً مقارنةً بالمجموعة الضابطة. كما أثبتت النتائج العلاقة الطردية بين تركيز المادة الحافظة والظواهر الحسية للعينات المعالجة؛ حيث حافظت عينات فيليه الدجاج المعالجة بـ ٥٠٠ جزء في المليون من مستخلص الشاي الاخضر على خصائصها الحسية لمدة تصل إلى عشرة أيام من التخزين المبرد. كما أظهرت النتائج انخفاضاً ملحوظاً في عدد بكتريا الكامبيلوباكتر في العينات المعالجة، حيث تناسبت نسب الانخفاض في عدد البكتريا تناسباً طرديا مع تركيز المادة المضافة على مدار فترة التخزين. ووفقًا لما سبق، يمكن التوصية باستخدام مستخلص أوراق الشاي الاخضر كمادة مضافة لتحسين الخصائص الظاهرية ومقاومة النموات البكتيرية في فيليه الدجاج اثناء التخزين المبرد.

**الكلمات الدالة:** مستخلص اوراق الشاي الاخضر، بكتريا الكامبيلوباكتر، حفظ لحوم الدجاج