Genotyping and Tetracycline Resistance of *Campylobacter jejuni & Campylobacter coli* Isolated from Broiler and Human Fecal Samples

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Abstract:
Campylobacteriosis is one of the main common food borne illnesses all over the world causing gastroenteritis in human. So, this study aimed to make genotyping of *Campylobacter* spp. isolated from fecal samples of both broiler and human with detection of tetracycline resistance strains. A total of 100 *Campylobacter* spp. previously identified and confirmed by presence of 23S*rRNA* gene from broiler and human fecal samples (50 of each) were subjected to duplex conventional PCR for detection of *mapA* gene which specific for *C. jejuni* and *ceuE* gene for *C. coli.* Tweenty randomly selected *Campylobacter* strains (10 from *C. coli* and 10 from *C. jejuni)* were examined for presence of *TetO* gene which responsible for tetracycline resistance. Results revealed that, the total percentages of *C. coli* and *C. jejuni* were 76% and 24% respectively. *C. jejuni* was 14 % while *C. coli* was 86% in fecal samples collected from broiler, while fecal samples collected from human, the percentage of *C. jejuni* was 34% while *C. coli* was 66%. All 20 recovered strains showed completely resistance to tetracycline with percentage 100%, which is considered the first record in Ismailia, Egypt. This study gives insight about the importance of continuous survey of *Campylobacter* strains with great attention to developing of newly antimicrobial resistance strains.

Keywords: *Campylobacter, ceuE* gene, *mapA* gene, *TetO* gene, Broilers, Human, PCR

Introduction:

Campylobacter is considered to be the most common bacterial cause of human gastroenteritis in the world ***(WHO, 2002).*** Consumption of undercooked poultry products or the mishandling of raw poultry products is the most likely source of exposure to *Campylobacter* ***(Bernadette et al., 2012).***

Broiler chickens are frequently asymptomatic carriers of *C. jejuni and C. coli* and the organisms are common contaminants of processed broiler carcasses. During the slaughtering and processing, poultry can become contaminated with *Campylobacter* from their intestinal contents ***(Burgos et al., 2017).***

The source of contamination has been attributed to the entry of *Campylobacter* into the processing plant in the intestinal tracts of asymptomatic broilers and the subsequent contamination of equipment and cross-contamination during processing ***(Park, 2002)***

The most important 2 types of Campylobacter spp. isolated from fecal samples in Portugal are *C. coli* and *C.jejuni*. *C. jejuni* is now the most widely recognized antecedent cause of Guillain–Barre´ syndrome, an acute paralytic disease of the peripheral nervous system ***(CDC, 2013).***

The incidence of *Campylobacter* was higher in case of fecal swab, followed by fecal samples then duodenum samples, while bile samples were negative. ***(Persson and Olsen 2005).***

C. coli showed higher resistance against antibiotics than *C. jejuni*. *C. jejuni* showed high resistance against ciprofloxacin and nalidixic acid ***(Schwan, 2010).*** Emerging of newly resistant *Campylobacter* isolates to antibiotics continuously occurs, so continuous survey of *Campylobacter* resistance should be under research.

Materials and Methods:

Samples

A total of 100 *Campylobacter* spp. previously isolated selective enrichment method using Bolton Broth and CCDA agar and well identified phenotypically. And genetically confirmed by detection of ***23SrRNA*** gene isolated from broiler and human fecal samples (50 of each) were subjected to duplex conventional PCR for genotyping and detection of *Campylobacter* tetracycline resistant strains at the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Ismailia branch, Egypt.

Extraction of DNA

According to QIAamp DNA mini kit instructions (Catalogue no. 51304). The QIAamp DNA Mini Kit provides silica-membrane-based nucleic acid purification from different types of samples according to ***(Ezzat et al., 2018)***. The spin-column procedure does not require mechanical homogenization, so total hands-on preparation time is only 20 minutes.

Genotyping of *Campylobacter* spp. using duplex Conventional PCR reaction

PCR Master Mix for cPCR was prepared according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310Akit as shown in Table (1). Primers were supplied from Metabion (Germany), they have specific sequence and amplify specific products as shown in Table (3) according to ***(Eunju and Lee***

2009). Cycling conditions of the primers during cPCR shown in Table (4).

Detection of *Campylobacter* resistant strains

About 20 randomly selected *Campylobacter* strains (10 strains from *C. coli* and 10 strains from *C. jejuni)* from both broiler and human were tested for detection of *TetO* gene which responsible for *Campylobacter* tetracycline resistance as the most common used antibiotic. PCR Master Mix for cPCR was prepared according to Emerald Amp GT PCR mastermix (Takara) Code No.RR310Akit as shown in Table (2). Primers were supplied from Metabion (Germany), they have specific sequence and amplify specific products as shown in Table (3) according to ***(Gibreel et al., 2004).*** Cycling conditions of the primers during cPCR shown in Table (4). Electrophoresis grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool at 70˚C, then 0.5µg/ml ethidium bromide was added and mixed thoroughly. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

Results:

Results shown in Table (5) revealed that the total percentage of *C. jejuni* isolated from both broiler and human was 24% while *C. coli* was 76 %. These results indicated that *C. coli* were represented in high percentage than *C. jejuni*.

The percentage of *C. jejuni* was 14 % while *C. coli* was 86%. While fecal samples collected from human, the percentage of *C. jejuni* was 34% while *C. coli* was 66%.

Photos (1) & (2) illustrated the genotyping of campylobacter strains isolated from broiler and human for detection of *mapA* gene for *C. jejuni* at 589 bp fragment and *ceuE* gene for *C. coli* at 462 bp fragment.

Regarding to sensitivity of randomly selected 20 campylobacter strains (10 from *C.coli* and 10 from *C.jejuni*) from both broiler and human fecal samples. Photo (3) showed that, all tested strains were resistant to tetracyclines with a percentage 100%

Table (1) Preparation of duplex PCR Master Mix for genotyping of *campylobacter* spp.

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	25*µl*
PCR grade water	15 *µl*
Forward primer(20 pmol)	1 *µl each (2)*
Reverse primer (20 pmol)	1 *µl each (2)*
Template DNA	6 *µl*
Total	50 *µl*

Table (2) Preparation of PCR Master Mix for tetracycline resistance gene:

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	12.5*µl*
PCR grade water	4.5 *µl*
Forward primer(20 pmol)	1 *µl*
Reverse primer (20 pmol)	1 *µl*
Template DNA	6 *µl*
Total	25 *µl*

Table (3): primers sequences for *ceuE, mapA and TetO* genes.

Target gene	specificity	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
ceuE	*C. coli*	AAT TGA AAA TTG CTC CAA CTA TG	462	**Eunju and Lee, 2009**
		TGA TTT TAT TAT TTG TAG CAG CG		
mapA	*C. jejuni*	CTA TTT TAT TTT TGA GTG CTT GTG	589	
		GCT TTA TTT GCC ATT TGT TTT ATT A		
TetO	Tetracycline resistance	GGCGTTTTGTTTATGTGCG	559	**Gibreel*et al*., 2004**
		ATGGACAACCCGACAGAAGC		

Table (4) Cycling conditions of the primers during cPCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
ceuE	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
mapA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
TetO	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Table (5) Percentage of C.coli and C. jejuni isolated from broiler and human fecal samples

Samples	No. of samples	C. coli		C. jejuni	
		No.	%	No.	%
Boiler	50	43	86	7	14
Human	50	33	66	17	34
Total	100	76	76	24	24

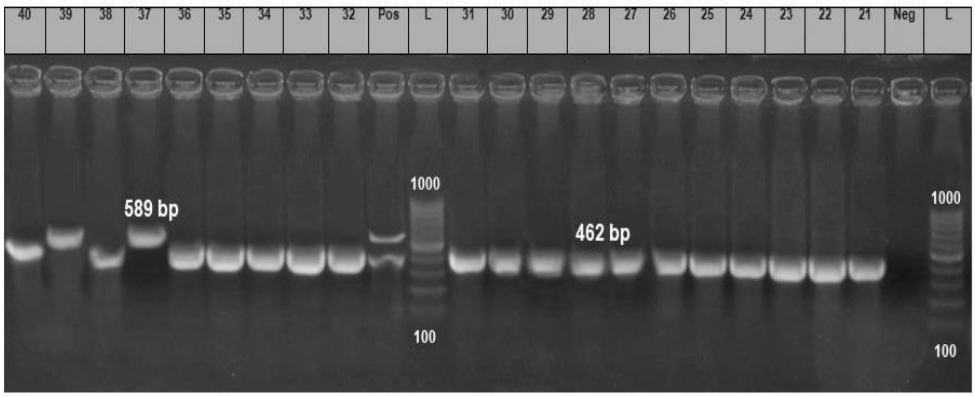

Photo (1): Agarose gel electrophoresis of 20 *Campylobacter* spp. isolated from broiler fecal samples for detection of *mapA* gene at 589 bp fragment and *ceuE* gene at 462 bp fragment. L: 100-1000 bp. Pos: positive control (*C. jejuni* and *C.coli* strains identified by RLQP). Neg: negative control (Master Mix without DNA). Lanes (from 21to 36, 38 and 40) positive for *C. coli.* Lanes (37 and 39) positive for *C. jejuni.*

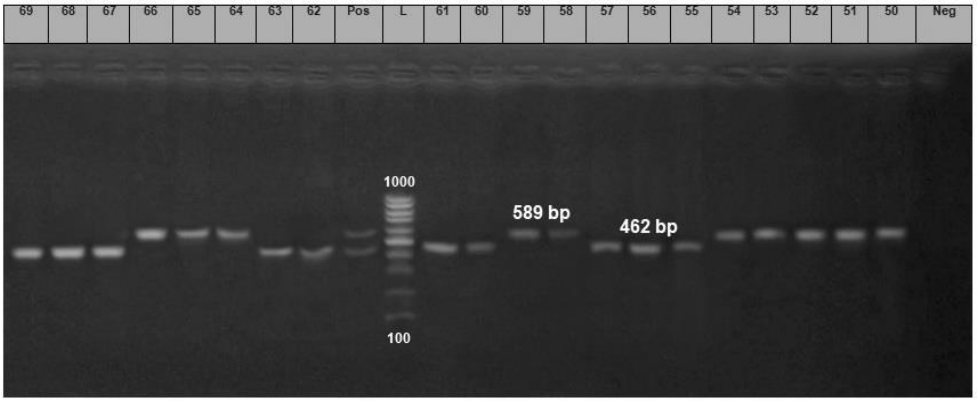

Photo (2): Agarose gel electrophoresis of 20 *Campylobacter* spp. isolated from human fecal samples for detection of *mapA* gene at 589 bp fragment and *ceuE* gene at 462 bp fragment. L:100-1000 bp. Pos: positive control (*C. jejuni* and *C. coli* strains identified by RLQP). Neg: negative control (Master Mix without DNA). Lanes (from 50 to 54, 58, 59 and from 64 to 66) positive

for *C. jejuni.* Lanes (from55 to 57, 60, 63 and from 67 to 69) positive for *C. coli.*

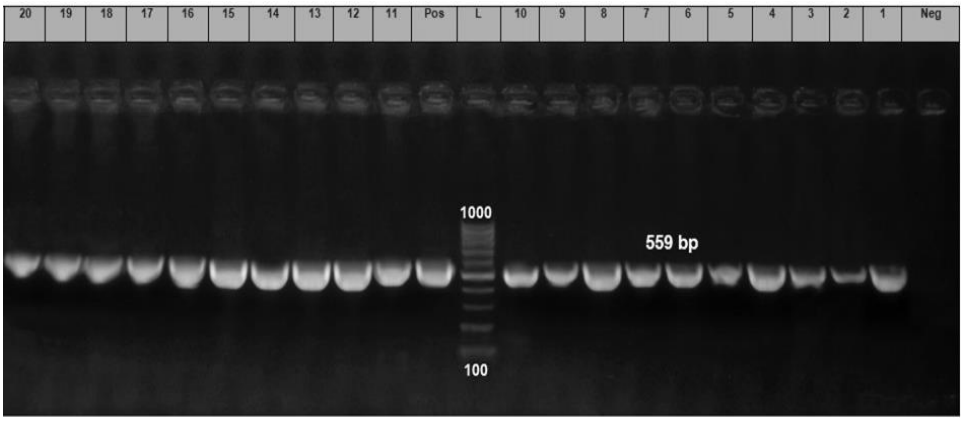

Photo (3): Agarose gel electrophoresis of 20 strains from *C.coli* and *C. jejuni* (10 for each) isolated from broiler and human fecal samples for detection of *TetO* gene for tetracycline resistance gene at 559 bp fragment. L: 100-1000 bp. Pos: positive control. (*Campylobacter* carrying *TetO gene*). Neg: negative control (Master Mix without DNA).

Discussion:

Results shown in Table (5) revealed that the percentage of *C. jejuni* isolated from broiler fecal samples was 14% while *C. coli* was 86%. These results nearly similar ***(Carreira et al., 2012)*** who obtained *Campylobacter* isolates with a percentage 83.3% in mainland Portugal, with a higher prevalence of *C.coli* (61.2%) than *C. jejuni* (38.8%). In Egypt ***(Sayed, 2000)*** examined 254 birds (190 visceral samples and 64 cecal swabs) on 4 broiler farms in for *Campylobacter spp.* and he found that 44 (23.1%) of visceral samples and 12 (18.7%) of cecal swabs were positive for *Campylobacter* spp. *C. jejuni* and *C. coli* were isolated from visceral samples and cecal swabs at the rate of (20.5%, 15.6%) and (2.6%, 3.1%) respectively. Of all isolates, 19.2% were *C. jejuni* and 2.7% were *C. coli*

Results discussed that isolation of both *C. coli* and *C. jejuni* from human fecal samples were 66% and 34% respectively, agreed with that obtained by ***(Butzler, 2004)*** who reported that cases of human campylobacteriosis represent only a small fraction of the actual number. Acute self-limited gastrointestinal illness, characterized by diarrhea, fever and abdominal cramps, is the most common presentation of *C. jejuni* and *C. coli* infection. ***(Nachamkin et al., 2000)*** stated that *C. jejuni* is now the most widely recognized antecedent cause of Guillain–Barre´ syndrome, an acute paralytic disease of the peripheral nervous system.

Regarding to, tetracycline resistant *Campylobacter* strains as shown in Photo (3). Which illustrated that all 20 randomly collected campylobacter strains were completely resistant to tetracycline as that all strains exhibited *TetO* gene. These findings agreed with ***(Wardak, 2005)*** who recorded that all isolated strains from *Campylobacter* showed high resistance to ciprofloxacin and erythromycin, while about 67% showed resistance to tetracycline, this is may occur due to mutation of *gyr*A protein gene ***(Charvalos et al., 1996).*** Tetracycline resistance has been reported due to presence of *Tet*O gene. *C. jejuni* fluoroquinolone resistant strains reported in Europe, Asia, and USA.

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الملخص العربى

تهدف هذه الدراسة إلى جعل التوصيف الجيني للكامبيلوباكتر المعزولة من عن عينات البراز لكل من الدجاج والإنسان مع تحديد مدى تواجد الجين الخاص بمقاومة التتراسيكلين. تم استخدام ١٠٠ معزولة من الكامبيلوبكتر سبق عزلها من عينات زرق الدجاج والبراز البشري (٥٠ لكل منهما) باستخدام CCDAوكذلك تم التوصيف الجيني من خلال وجود جين ***٢٣SrRNA*** . تم عمل اختبار تفاعل انزيم البلمرة المتسلسل لجميع العينات للكشف عن كل من***mapA*** الذي يخص جينات كامبيلوباكتر جيوجناى و ceuE كامبيلوباكتر كولاى وكذلك عن جين ***TetO*** المسؤول عن مقاومة التتراسكلين. أظهرت النتائج أن إجمالي النسب المئوية لـ ميكروبى كامبيلوباكتر كولاى و كامبيلوباكتر جيوجناى كانت ٧٦% و ٢٤% على التوالي. كانت نسبة توجد ميكروب كامبيلوباكتر جيوجناى (% 14) بينما كان كامبيلوباكتر كولاى (86%) في عينات البراز التي تم جمعها من زرق الدجاج ، في حين أن عينات البراز التي تم جمعها من الإنسان ، كانت نسبة كامبيلوباكتر جيوجناى كانت ١٤% بينما كانت كامبيلوباكتر كولاى ٦٦%. أظهرت جميع السلالات العشرين التى تم فحصها مقاومة تيتراسيكلين تمامًا بنسبة ١٠٠٪ ، والتي تعد أول رقم قياسي في الإسماعيلية ، مصر. تعطي هذه الدراسة مؤشر مبدأى حول أهمية المسح المستمر لسلالات Campylobacter مع الاهتمام الكبير بتطوير سلالات مقاومة جديدة للميكروبات.