Comparative Study Between Traditional Methods, Commercial Biochemical Test and PCR for Identification of *Campylobacter* Species Isolated from Poultry Farms

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Abstract:

Campylobacter spp. causes severe issues in chickens. In this investigation. *Campylobacter* species were isolated (182) in Dakahlia Governorate's from 440 broilers and 440 ballade breeds. *Campylobacter* coli 42 isolates (4%)and *Campylobacter jejuni*140isolates (16%). For *C. jejuni*, rates of recovery from various internal organs were 60%, 24%, 10%, and 5%, from the cloacal swab, heart, liver, and gizzard respectively. The morphological studies revealed that the majority of species from *Campylobacter* are motile, survive at 37-42C^{o}, and are G-ve, slender spirally curled. They require 5%O2, 85%N2 and 10%CO2 to create a colony that looks like a dew drop on mCCDA media. By using API20, the suspected isolates were identical to typical *Campylobacter*

Keywords*: Campylobacter jejuni, C. coli*., Poultry, API 20E and PCR.

Introduction:

Campylobacter spp. were acknowledged as among the most frequent causative organisms of enteritis and, human gastroenteritis ***(Caprioli A et al., 1996*)**. *Campylobacteriosis* cases now outnumber those brought on by traditional intestinal bacteria. The rate of detection of *Campylobacter* spp. from patients with infections of alimentary tract was 3-4 times higher than *Escherichia coli* and *Salmonella* (***EFSA, 2017)***. *Campylobacter* infection rates have risen recently (**Platts et al., 2014**).

Family *Campylobacteriaceae* includes 22 species, where *C. coli* and *C. jejuni* are the primary cause of human gastroenteritis, despite other, "developing" species like *C.* concisus, *C.* hyointestinalis, C.

upsaliensis, C. sputorum and C. ureolyticus, were responsible for gastroenteritis and periodontitis **(Fitzgerald and Nachamkin, 2011)**. Typically, diverse animal species, whether domestic or wild, become colonised by *Campylobacter* species, which are also present in food of animal origin (**Man, 2011).**

Campylobacter species could grow at temperatures ranged from 37° - 42° C with a pH ranged from 6.5 - 7.5. So they defined as "thermophilic". However, **(Levin 2007**) reported that these bacteria do not exhibit a true thermophilia and cannot thrive at temperatures equaled to or above 55° C, they are more appropriately referred to as "thermotolerant.".

Aim of the work:

This work was done to identify and isolate *Campylobacter spp*. from poultry with traditional, commercial biochemical tests and using PCR to identify the most reliable *Campylobacter* Spp isolated from poultry.

Material and Methods: -

For isolation and identification of *Campylobacter species*, cloacal swabs and organ samples (heart, gizzard, and liver) from 110 apparently healthy Ballade breeds and 110 broilers chicken's samples from poultry farms located at Dakahlia Governorate were collected. To avoid cross contamination, all samples were handled aseptically with sterile sampling equipment. and promptly carried in an ice box to the lab. for further bacteriological examinations. In sterile tubes, a loopful of each sample was cultivated for 24 to 72 hours onto Thioglycollate broth medium, Then, a loopful from each tube was streaked on a special antibiotic-infused Blood-free selective medium for *Campylobacter*. The inoculated plates were kept in anaerobic jars for. with kits that generate CO2, O2, and nitrogen at a temperature of 37 °C for 48 hours, and the motility of the bacteria was then observed under a phase contrast microscope.

The suspicious colonies were then purified on blood agar medium supplied defibrinated sheep blood for 24 hours. **Oyarzabal and Battie (2012)** suspected colonies were carefully examined for their morphological characteristics according to **ISO (2006).**

Biochemical identification:

A.Catalase production test

A positive results represented by bubbles formation due to release of O_2 from the H_2O_2 in the presence of catalase. (*C. jejuni, C. coli*) (**ISO, 2006).**

B.*Hydrogen sulphide production*:

(Bailey & Scott's.2007)

Blackening of the medium mean H_2S production.

C. *Hippurate hydrolysis test*.

used to distinguish between C. jejuni and C. coli

commercial kits (API20E):

API 20 E is a standard kit that is designed for the identification of bacteria that belong to the family *campylobacteriaceae* and It cannot be used to detect the absence or presence of any other microorganisms.

Identification is obtained with the numerical profile.

Determination of the numerical profile:

The tests are divided into groups of three on the result sheet, and a value of 1, 2, or 4 is listed for each. By summing the values corresponding to favorable responses in each group, for each of the 20 tests of the API 20 E strip, a 7-digit profile number is obtained. The oxidase reaction makes up the twenty first test and, in the event that it is affirmative, has a value of 4.

Finally, these seven - digit profile number for each strip were used for identification with identification software or the analytical profile index.

In some cases, this seven - digit profile number was not enough and supplementary test need to be carried out by nitrate reduction and used to be formed eight - digit profile number.

Molecular detection of *campylobacter* spp. common genes:

Conventional PCR assay for *Campylobacter* isolate confirmation was performed. DNA was obtained through a QIA amp DNA mini kit (Germany). An Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit. was used to prepare the PCR Master Mix also the cycling conditions for primers during PCR. In PCR, oligonucleotide primers with specific sequences amplify a particular product. are shown in Table (1) and the cycling conditions of each primer are showed in table (2).

Table (1). *Sequences of oligonucleotide primers.*

Target gene	Primersequence (5'-3')	Lengthof amplified Product	Reference
23S rRNA	TATACCGGTAAGGAGTGCTGGAG	650bp	Wang *et al*., 2002
	ATCAATTAACCTTCGAGCACCG		
C. jejuni hipO	GAA GAG GGT TTG GGT GGT G	735bp	Al Amri *et al*., 2007
	AGC TAG CTT CGC ATA ATA ACT TG		
C. coli asp	GGT ATG ATT TCT ACA AAG CGA G	500bp	
	ATA AAA GAC TAT CGT CGC GTG		

Table (2) Primers cycling conditions during cPCR.

Gene	Primary denaturation	Secondary Denaturation	Annealing	Extension	No. of cycles	Final extension
23SrRNA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
C. coli (asp)	94˚C 5min	94˚C 3sec.	49˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
C. jejuni (hipO)	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 50 sec.	35	72˚C 10 min.

Results:

Prevalence of *Campylobacter* spp in poultry: -

A total of 182 *Campylobacter* spp. isolates were recovered out of 880 samples. The isolation rate of C. *jejuni* was 14% in broilers (65/440) and 17% in ballade breeds (75/440); this show that *C. jejuni* in ballade breeds was higher than in broilers breeds. The isolation rate of *C. coli* was 5% in broilers (22/440) and 4.5% in ballade breeds (20/440). According on the organ of sampling, there was a statistically significant variation in the presence of Campylobacter in chickens. The highest rate of *C. jejuni* and *C. coli* from different organs recovered from cloacal swaps followed by the heart, liver then gizzard in both broiler and Ballade breeds (Table 3 and 4).

Identification of *Campylobacter* isolates:

On charcoal-based surfaces like m CCDA, the typical colonies have a tendency to spread and are greyish, flat, wet, and may have a metal sheen is characteristic for *C. jejuni,* while *C. coli* appear as colonies of a creamy-grey, wet, and more distinct form. The isolated colonies were catalase, H_2S positive, while Hippurate was positive with *C.jejuni* and negative in *C.coli*

Result of commercial kits (API20E):

As shown in figure (1) and (2)

Molecular confirmation of campylobacter DNA

Ten isolates were subjected to molecular examination for common gene *(23srRNA)* . The results showed the presence of *campylobacter* DNA in the 9 isolates by using *23srRNA* gene at 650 bp with percent 100% as detected in fig (3). *C.jejuni* and *C.coli* that previously identified by morphological methods were confirmed by specific primers for both species (*hipO* and *asp* respectively) as shown in figure (4,5)

Table (3) *Recovery pattern of C.jejuni from positive samples*

Type of Birds	Number of +ve samples	Cloacal swab NO %		Heart NO %		Liver NO %		Gizzard NO %	
Broilers	65	40	61	14	21	7	10	4	6
Ballade	75	44	58	20	26	8	10	3	4
Total	140	84	60	34	24	15	10	7	5

Table (4) *Recovery pattern of C. coli from positive samples*

Type of birds	No of +ve samples	Cloacal samples NO %		Heart NO %		Liver NO %		Gizzard NO %	
Broilers	22	9	40	8	36	3	14	2	5
Ballade	20	10	50	5	25	3	15	2	10
Total	42	19	45	13	30	6	14	4	10

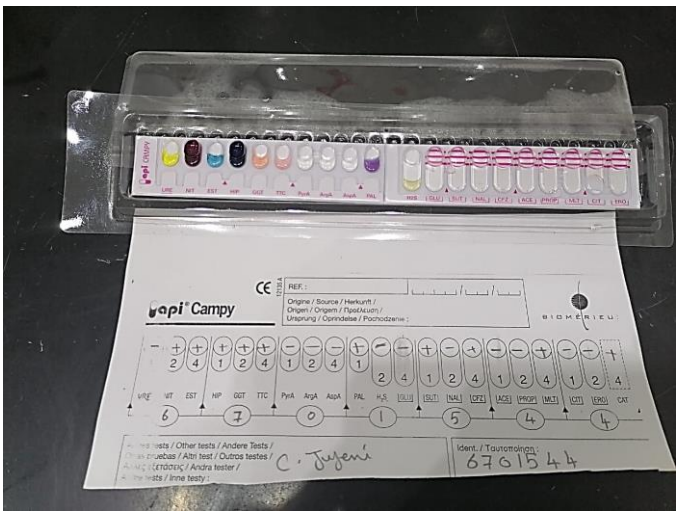

Figure (1) Biochemical identification of *C.jejuni* by using API20E

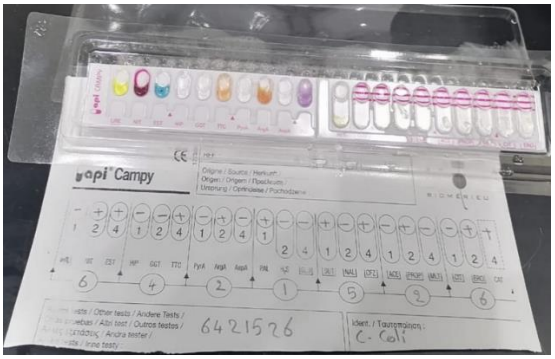

Figure (2) Biochemical identification of *C.Coli* by using API20E

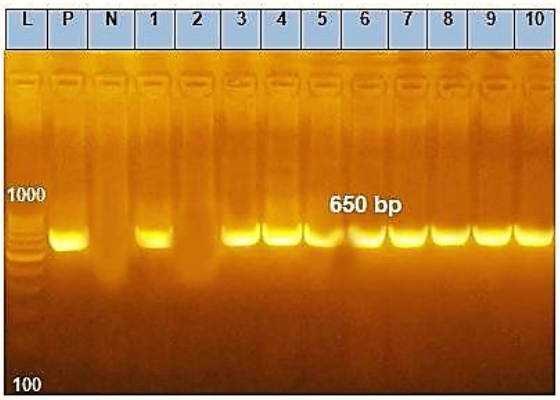

Figure (3) detection of *23srRNA* gene (common gene) at 650 bp

figure (3) *23Sr RNA* primer-based amplification of a 650 bp fragment on an agarose gel. (common gene of *campylobacter*, L: 100-1000bp ladder, Lane (pos.): Positive control (*Mycoplasma*). Lane (neg.): Negative control (Saline), and Lane (1and 3-10): Positive samples (*Campylobacter spp.)*

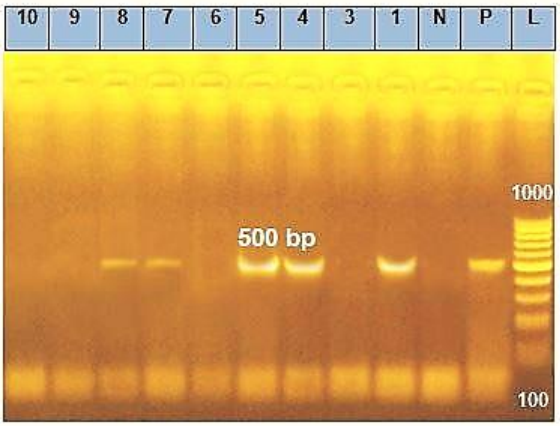

Figure (4) detection of *asp* gene was presented in all campylobacter coli isolates with percentage (100%)

Figure (4) Agarose gel electrophoresis demonstrating *asp*-based 500 bp fragment amplification. (specific gene of *C. coli)* L: 100-1000bp ladder.
Lane (pos.): Positive control (*mycoplasma).* Lane (neg.): Negative control (Saline).
Lane (1,4,5,7 and 8): Positive samples *(Campylobacter coli.)*

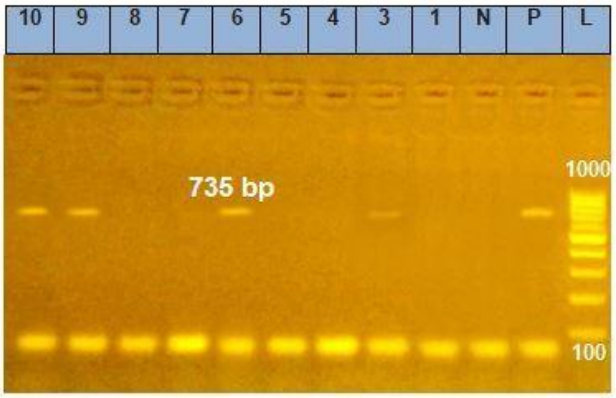

Figure (5) detection of *hipO* gene was presented in all campylobacter Jejuni isolates with a percentage (100%)

Figure (5). Agarose gel electrophoresis showing amplification of 735bp fragment using *hip O* primer. (Specific gene of *C. jejuni)*
L: 100-1000bp ladder.
Lane (pos.): Positive control (*mycoplasma*). Lane (neg.): Negative control (Saline).
Lane (3,6,9 and 10): Positive samples (*Campylobacter jejuni.)*

Discussion:

According to epidemiological research, handling and consuming raw or undercooked poultry items can cause up to 50–80% of all human *Campylobacter* infections.

A 1 Log10 CFU/g decrease in the quantity of According to the ***European Food Safety Authority (EFSA) (2011),*** campylobacter was found on carcasses. could potentially cut the danger to the public health by 50 to 90%.

Similar to the findings of a surveillance research conducted in England and Wales, compared to *C. coli*, *C. jejuni* was discovered in more than 12 times the number of instances of human illness according to ***Friedman et al. (2000).*** According to ***Gillespie et al. (2002),*** who established comparable findings to those of Friedman et al. (2000), 93% of human sickness was caused by *C. jejuni*, and the majority of the

remaining cases were caused by *C. coli*. Because of this, the majority of research and studies have looked at the prevalence and physiological traits of *C. jejuni* and *C. coli*, two representative *Campylobacteraceae* organisms.

The present study was comparable to the outcome reported by ***(Nahed et al., 2021)*** 20% and 17.75%, respectively, C. *jejuni* were found in of the intestinal contents of laying and broiler chickens, this predominance may be related to the fact that several *Campylobacter* species are thought to be more prone to the digestive system of hens, particularly the caecum and colon ***(Jokinen et al., 2011).***

Conclusion:

Poulty meat is the leading source of animal protein for human consumption in many countries. biochemically identified *Campylobacter* were molecularly confirmed by the amplification of *23S rRNA*(common gene of campylobacter ,*hipO gene* (specific gene *of C.jejuni*), *aspgene* for C.Coli

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الملخص العربي

الكامبيلوباكتر هي مشكلة صحية هامة وتحديا كبيرا في جميع انحاء العالم . يتم التعرف علي انواع الكامبيلوباكتر كمسبب للاسهال حيث أن ميكروب الكامبيلوباكتر موجود في الجهاز الهضمي في الدواجن
. وينتقل الي الانسان فيسبب الاسهال وتجرثم الدم والتهاب المفاصل ومتلازمة غيلان باربيه والاضطرابات العصبية . تعتبرالدواجن من المصادر الهامة للعدوى البشرية الدواجن
الكامبيلوباكتر هي سالبة الجرام ,حلزونية لها سياط أحادية او ثنائية وتضم أكثر من 10أنواع أكثرها شيوعا كامبيلوباكتر كولي وكامبيلوباكتر جيجوني .
يمكن أن تقلل من انتشار استعمار الكامبيلوباكتر بالقرب من عمر السوق من 40 إلى80٪ من خلال الحواجز الفيزيائية والكيميائية. يمكن أن يساهم تنظيف وتطهير بيوت الدواجن بين القطعان بشكل فعال في الحد من استعمارالكامبيلوباكتر. علاوة على ذلك ، التدابير الصحية الصارمة والقيود العامة على الأمن البيولوجي.
ولذلك تهدف هذه الدراسة الي تحديد الاصابة بهذا المرض الضار وكيفية عزل الميكروب المسبب له ولتحقيق هذا الهدف قد تم القيام بهذه الخطوات :-
1- تم عزل 182 عترة من الكامبيلوباكتر من 440 دجاجة من بداري التسمين و440 دجاجة بلدي من محافظة الدقهلية بنسبه 16%(140) كامبيلوباكتر جيجوني 4%(42) كامبيلوباكتر كولي .
2- أظهرت معدل عزل الكامبيلوباكتر من الأعضاء الداخلية المختلفة بنسبة عالية من محتويات الأمعاء الداخلية والقلب والكبد والقونصة بنسبة 60%و 24% و10%ثم 5% لكامبيلوباكتر جيجوني وايضا بنسبة 45% &30% &14% ثم 4% لكامبيلوباكتر كولي .
3- أظهرت نتائج التصنيف لأنواع الكامبيلوباكتر المعزولة من الدواجن تواجد كامبيلوباكتر كولي بنسبة 16% ممثلة في 140 عينة وكامبيلوباكتر جيجوني بنسبة 4% ممثلة في 42 عينة .
4- تم اجراء الاختبارات المورفولوجية التي اثبتت ان ميكروب الكامبيلوباكتر سالبة الجرام &اسطوانية وغالبيتها متحركة تنمو عند درجة حرارة 37-42 درجة مئوية وتحتاج 15% أكسجين &10% ثاني اكسيد الكربون &85% نيتروجين .
5- تم اجراء تفاعل البلمرة المتسلسل باستخدام البادئ المتخصص لكل جين من جيناتت الضراوة الاكثر شيوعا وهم (hipO&asp)
وقد تم في البداية اجراء اختبار تفاعل البلمرة المتسلسل باستخدام البادئ العام للكشف عن جين 23SRN وقد تبين تواجده بجميع معزولات الكامبيلوباكتر .