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### Original Paper

## Phenotypic and genotypic characterization of *Campylobacter* isolated from poultry

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### ABSTRACT

Campylobacteriosis incidence and proportion of *Campylobacter* strains resistant to antibiotic have been increased worldwide in the last decades. Campylobacteriosis is considered as one of the major important zoonotic gastrointestinal diseases around the world. The aim of this study was isolation and biochemical characterization of *Campylobacter* from poultry. *Campylobacter* isolation have been done on MCCDA media, motility has been detected by phase contrast microscope, morphology has been detected by Gram stain, and confirmed by MALDI-TOF MS and molecular confirmation by using cPCR by amplification of 16S rRNA gene. A total of 102 isolates of *Campylobacter* were isolated from farm and small backyards located in different sources in El-Kalyobia, El-Monofia and El-sharkia Governorates by conventional bacteriological methods from which 40 were *C. jejuni* and 62 were *C. coli*. All isolates have been confirmed as *Campylobacter* by MALDI-TOF MS and detection of 16S rRNA by cPCR. PCR is a useful molecular tool for identification and confirmation of *Campylobacter*. It is rapid, sensitive, and specific than the culture methods, but the only disadvantage is that is expensive.

## 1. INTRODUCTION

One of the major important zoonotic gastrointestinal disease is Campylobacteriosis around the world, and *Campylobacter jejuni* (*C. jejuni*) is the most prevalent causative agent. Poultry has a significant role in transmission of Campylobacteriosis to human (Gormley *et al.*, 2008).

Thermophilic *Campylobacter* species have Gram negative cell wall structures with capsule and flagella. The bacteria are slender, curved rod to small spiral in shape with 0.2-0.5 mm width and 0.5-5.0 mm length (Shane and Harrington, 1998). *C. jejuni* is the major microorganism of food borne *C. enteritis* in human, followed by *C. coli* and then *C. lari* (Skirrow and Blaser, 2000).

Campylobacteriosis incidence has been increasing worldwide during the last decades (Luangtongkum *et al.*, 2009) leading to a rise of public health disease. The number of diseased persons and death rate were both expanded (Euro Surveill., 2015).

Contaminated broilers represent the highest risk for consumers (Sahin *et al.*, 2015). In comparison, layers have not got equivalent consideration, layers might be the main reservoirs for antimicrobial-resistant *Campylobacter*. This is critical because layer farming is a gigantic operation in Egypt. Thusly, these hens that carry antimicrobial resistant *Campylobacter* can pose a potential risk of environmental contamination and subsequent transmission through farm animals, wildlife and workers (Ahmed *et al.*, 2013).

Particularly, *Campylobacter* can stay in feces of layers and in poultry litter for a few days, which proposed that the land utilization of these byproducts as fertilizers might contribute to the scattering of the pathogens (Kassem *et al.*, 2010; Ahmed *et al.*, 2013).

*Campylobacter* diagnosis is obstructed by the fastidious characteristics of these microorganisms, and it can become more difficult by appearance of a viable-non-cultivable state of these microorganisms (Jackson *et al.*, 2009).

The purpose of the current study is to describe cultivation and identification of thermophilic *Campylobacter* from poultry by a combination of bacteriological and molecular methods.

## 2. MATERIAL AND METHODS

### 2.1. Samples:

The present study was conducted during the period of January 2016 to September 2019 on a total of 617 sample from 38 poultry farms and small backyards located in different sources in El-Kalyobia, El-Monofia and El-sharkia Governorates.

### 2.2. Isolation of *Campylobacter* species:

Loopfuls from each sample were cultured directly onto thioglycolate broth medium for 24-72 hours in sterile tubes, then a loopful from each tube was cultured on modified *Campylobacter* blood free selective medium with

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antibiotics. All inoculated plates were incubated in anaerobic jar with kits which generates CO<sub>2</sub> (10%), O<sub>2</sub> (5%) and N<sub>2</sub> (85%) at 37 °C for 48 hours and were demonstrated daily for the characteristic colonies. Then purification of the suspected colonies on blood agar media with defibrinated sheep blood containing Campylobacter growth supplement for 24 hours.

### 2.3. Morphological identification of Campylobacter isolates:

Suspected growing colony on the specific agar plates were examined carefully for their morphological characters according to Koneman et al. (1995). A single suspected colony was stained with Gram's stain to demonstrate the characteristics morphology of the isolates. Campylobacter species are Gram negative.

### 2.4. Motility:

Direct smear from 3 days old culture were examined under phase contrast microscope to demonstrate the corkscrew like motion characteristic to Campylobacter species (Smibert, 1974)

### 2.5. Biochemical identification:

The purified colonies were identified biochemically by catalase production test. A small amount of pure growth were placed onto the surface of a clean, dry glass slide by sterile loop then a drop of 3% hydrogen peroxide was added into a portion of colony on the slide (Laing, 1960), production of bubbles of gas, indicating the production of catalase enzyme. Oxidase activity was examined with 1% aqueous solution on filter paper of tetramethyl-p-phenyldiamine-dihydrochloride as a reagent. With a wooden loop a separate well grown colony will be picked up from a fresh culture medium (24 hours) and applied to the reaction on the filter paper (El-Gohary, 1998). A positive reaction was indicated by a violet coloration within 20-60 seconds at the contact point.

### 2.6. Identification by MALDI-TOF MS:

Identification of isolates by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bizzini et al., 2010). After centrifugation for 5 min at 10.000 ×g, the supernatant was removed and the pellet was re-suspended in 50 µl of 70% (vol/vol) formic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Fifty microliters of acetonitrile (Carl Roth GmbH) were added, mixed and centrifuged. One and a half microliter of the supernatant was transferred onto MTP 384 Target Plate Polished Steel TF (Bruker Daltonik GmbH, Bremen, Germany). After air-drying the material was overlaid with 2 µl of a saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich Chemie GmbH). After air-drying spectra were acquired with an Ultraflex (Bruker Daltonik GmbH).

Analysis was carried out with the Biotyper 3.1 software (Bruker Daltonik GmbH). Interpretation of results was performed according to the manufacturer's recommendation: score of ≥ 2.3 represented reliable species level identification; score 2.0–2.29, probable species level identification; score 1.7–1.9, probable genus level identification, and score ≤ 1.7 was considered an unreliable identification (Lüthje et al., 2017).

### 2.7. Molecular confirmation of Campylobacter isolates:

Genomic bacterial DNA was prepared from colonies with typical growth and subculture on blood agar. The samples were processed according to the manufacturer instructions by using the High Pure PCR Template Purification Kit (Roche Diagnostics, Mannheim, Germany). PCR amplifications were carried out targeting 16S rRNA gene (S1: ATC TAA TGG CTT AAC CAT TAA AC, S2: GGA CGG TAA CTA GTT TAG TAT) as described by Denis et al. (2001). Gel electrophoresis used for analysis of PCR products on 2% agarose gels following staining with ethidium bromide and visualized under UV light

## 3. RESULTS

### Identification of thermophilic Campylobacter

A total of 102 Campylobacter species were identified from different poultry species by bacteriological examination by their characteristic colony on mCCDA and oxidase production as thermophilic Campylobacter produced intense deep purple color appearance within few seconds on oxidase strip, by gram stain it was appeared as gram negative twisted bacilli and Campylobacter showed there characteristic cork screw motility when examined by phase contrast microscope, MALDI-TOF revealed the same result.

Table1 Occurrence of Campylobacter jejuni and coli in poultry samples

Poultry species	no.	Positive <i>Campylobacter spp.</i>		<i>Campylobacter r jejuni</i>		<i>Campylobacter coli</i>	
		No.	%	No.	%	No.	%
Layers	24	32	13.1	10	31.2	22	68.7
	4						
Broiler	21	36	16.9	16	44.4	20	55.5
	3						
Turkeys	32	9	28.1	2	22.2	7	77.7
Ducks	12	25	19.5	12	48.0	13	52.0
	8						
Total	61	102	16.5	40	39.2	62	60.7
	7						

no.: number of samples. Broiler: Broiler chicken.

### Confirmation of campylobacter isolates by conventional PCR:

Accurately, 102 Campylobacter isolates were confirmed by amplification of 16S rRNA gene at size of 857 bp using cPCR (Figure 1).

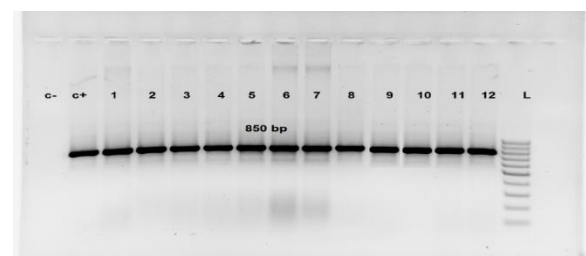


Fig. 1 Typical amplification of 16S rRNA gene. Lane L: 100-1000 bp DNA Ladder. C-: Negative control. C+: Positive control. Samples from 1 to 12: Campylobacter (positive) at 850 bp

## 4. DISCUSSION

In the last ten years, the occurrence of Campylobacteriosis were in an increasing trend. As *C. jejuni* able to colonize poultry intestine that lead to poultry meat a reservoir of foodborne Campylobacteriosis (Ayaz et al., 2016). The main route of transmission of infection is eating of food of animal

origin and particularly poultry meat (Butzler and Oosterom, 1991).

Differentiation of *C. jejuni* and *C. coli* traditionally relied on the hippurate hydrolysis test; *C. jejuni* hydrolyses hippurate whereas *C. coli* does not.

Polymerase chain reaction has become a reliable alternative to the traditional biochemical method of detection. PCR can identify bacteria at the gene level and this assay has been used for the detection of *Campylobacter* in poultry (Giesendorf et al., 1992; Hazeleger et al., 1994). Isolation and identification of *Campylobacter* spp have traditionally involved the use of selective culture media combined with biochemical tests. This method is expensive, laborious and time consuming, whereas PCR is cheaper and nearly 4 times faster than SCM. In recent years, PCR has increasingly been applied in detection and identification of *Campylobacter* spp. Several reports used PCR method have shown great improvement in accuracy and sensitivity, associated with fast sample processing (Englen and Fedorka-Cray, 2002; Wang et al., 2002).

In the current study, *Campylobacter* spp. were isolated from 16.5% cloacal swabs from all poultry species, near results were obtained by Awadallah et al. (2014), Carrique-Mas et al. (2014), Torralbo et al. (2014), Mäesaar et al. (2014) and Sabike et al. (2017) their rates were 21.6% 31.9%, 38.1%, 39.2% and 29%, respectively. However, other study by Messad et al. (2014) and Da Silva et al. (2016) reported higher prevalence rates of *Campylobacter* spp. 85% and 58%, respectively.

The recorded data showed that *Campylobacter* species which isolated from chicken samples (broilers & layer chicken) revealed that *Campylobacter* spp. were 32/244 (13.1%) from layers and 36/213 (16.9%) from broiler. This result in agreement with Hofshagen and Kruse (2005) (4.8%), Stern et al. (2005) (17.6%-12.7%) and Bai et al. (2014) (26.3%).

According to turkeys, the prevalence of *Campylobacter* was (28.1%) this nearly like the values reported by other authors Logue et al. (2003) (34.9%), whyte et al. (2004) (37.5%), Arsenault et al. (2007) (46%), Little et al. (2008) (33.7%), cook et al. (2009) (46%), Moran et al. (2009) (56%) and Perko-Makela et al. (2009) (31.4%).

The obtained results revealed 25/128 (19.5%) *Campylobacter* species in ducks which was like results obtained from ducks in Thailand Boonmar et al. (2007) (20%) and Hamed et al. (2014) (24%). While *Campylobacter* in this study was lower than whyte et al. (2004) (45.8%), Tsai and Hasing (2005) (43.5%) and Rahimi et al. (2011) (35.5%) and higher than Nor et al. (2013) (12%).

The high prevalence of *Campylobacter* in poultry in our study explained by the reason that poultry is exposed to campylobacters at farm due to low level of biosecurity and hygiene measures, and thus lead to contamination of carcasses during different slaughtering processes and so transmission of infection to human.

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