Bacteriological and Molecular Identification of some *Campylobacter* Species in Broilers and their Macrolide Resistance Profile

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A B S T R A C T

Genus campylobacter including several species is of great importance that is considered among the major causative agents of acute diarrheal diseases in humans worldwide. The current study was carried out to determine the occurrence of thermotolerant campylobacters in broilers and to identify the macrolides resistance profiles of *C. jejuni* and *C. coli* isolates. A total of 568 samples (364 cloacal swabs and 51 of each breast meat, thigh meat, caecal part and neck skin) were collected from broiler chickens at slaughter age from local pluck shop outlets in Zagazig city, Sharkia Governorate, Egypt. The isolation rate of *Campylobacter* species from neck skin, breast meat, cloacal swabs, thigh meat and caecal parts samples was 25.5%, 27.5%, 29.3%, 31.4% and 41.2%, respectively. *C. jejuni* was isolated from cloacal swabs, skin, thigh meat, breast meat and caecal parts samples with the isolation rate of 55.3%, 53.8%, 43.7%, 50% and 80.9%, respectively. Forty-two campylobacter isolates (28 and 14 biochemically suspected *C. jejuni* and *C. coli* isolates, respectively) were confirmed molecularly depending on 23S rRNA gene. Furthermore, real time PCR targeting *hip*O gene specific for *C. jejuni* and *gly*A specific for *C. coli* were used. The molecularly confirmed isolates were evaluated they macrolides resistance pattern which revealed that all isolates were resistant to macrolides. Further studies on the mechanisms of macrolides resistance in campylobacters are essential.

Keywords: *C. jejuni*, *C. coli*, broiler, rtPCR, macrolide, Egypt

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1. INTRODUCTION

Campylobacter is considered an emerging foodborne disease (EFSA and ECDC, 2015), which was recognized as a major cause of human illnesses ranging from gastroenteritis to sever illness; Guillain-Barre Syndrome (Moore et al., 2005).

Poultry is a natural host of *Campylobacter* spp. and the broiler chicken gut especially the caeca is often colonized by *C. jejuni* in particular (EFSA, 2008). *Campylobacter* spp. are Gram-negative,

curved rods within the family campylobacteriacae (Zilbauer et al., 2008), which require specific growth conditions for an optimal growth (Bronnec et al., 2016).

Amongst campylobacters, thermophilic *Campylobacter* species are implicated in food borne infections (Iovine et al., 2008). *C. jejuni* and *C. coli* are recognized as the major cause of acute gastroenteritis in human throughout the world. *C. jejuni* is most frequently reported as a cause of human campylobacteriosis (80-90%) compared to *C. coli* (5-10%) (EFSA, 2008).

Hippurate hydrolysis is the only phenotypic test differentiating *C. jejuni* from other species of campylobacters, especially the thermophilic species. The two biotypes of *C. jejuni* (*C. jejuni* subspp. *jejuni and* subspp. *doylei*) are capable of hydrolyzing sodium hippurate to benzoic acid and glycine (ISO, 2006). However, recently, hippurate negative *C. jejuni* strains have been reported in several studies (Waino et al., 2003). For instance, Takkinen et al. (2002), documented 2.5% false positive hippurate hydrolysis results and 39% false negative results.

Gastroenteritis caused by campylobacter infection is usually self-limiting and require no antimicrobial treatment except in severe and immunocompromised patients (Belanger and Shryock, 2007). Fluoroquinolones (FQ) and macrolides are the most commonly used antibiotics in the treatment of campylobacter infections (Da Silva et al., 2016). However, the rapid significant increase in the prevalence of FQ and macrolides resistant strains isolated from broiler sources (Neimann et al., 2003), is recognized as the major health problem worldwide (Griggs et al., 2005). The increased resistance has been reported due to the un-controlled use of antibiotics especially in poultry industry (Chang et al., 2015).

The aim of the current work was to investigate the prevalence of *Campylobacter* species particularly *C. jejuni* in broiler chicken samples by using conventional and molecular tools. Also, identifying the macrolides resistance profile of *C. jejuni* and *C. coli* isolates using both minimum inhibitory concentration and disc diffusion methods.

2. MATERIAL AND METHODS

2.1. Samples:

A total of 568 samples were collected from broiler chickens at slaughter age (6 weeks) from local pluck shop outlets in Zagazig city, Sharkia Governorate, Egypt. The samples comprised of 364 cloacal swabs and 51 of each breast meat, thigh meat, caecal part and neck skin. The study was carried out during the period from September 2015 to July 2017. The collected samples were aseptically transported as soon as possible in an ice box to the laboratory for bacteriological examination.

2.2. Samples preparation:

2.2.1. Poultry cloacal swabs:

Sterile swabs were inserted into the cloaca, and then directly immersed into tubes containing sterile preston enrichment broth medium (Ellerbroek et al., 2010).

2.2.2. Poultry skin, meat and caecal samples:

Twenty five grams of each breast meat, thigh meat, incised skin and caecal

samples were aseptically transferred to a sterile blender containing 225 ml of preston enrichment broth for homogenization then enriched (Sallam, 2001).

2.3. Bacteriological examination:

2.3.1. Isolation of Campylobacter species:

For isolation of *Campylobacter* species, the collected samples in preston enrichment broth were incubated at 42ºC for 24-48 hours with less than 1 cm of headspace left in the culture vessel with tightly capped lids (Oxoid, 2006). After enrichment, 0.1 ml of the broth was streaked onto modified campylobacter selective agar; mCCDA containing CCDA selective supplement. The plates were then incubated at 42ºC in darkness for 48 hours under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) using CampyGen sachets (Vandepitte and Verhaegen, 2003).

2.3.2. Preliminary confirmation of thermophilic Campylobacter species

Thermophilic Campylobacter species were preliminary identified by their colonial morphology on mCCDA media. Suspected colonies were purified on 5-7% lysed horse blood agar plates and subjected to Gram staining, testing of motility, growth at 25ºC and 41.5ºC and oxidase test (ISO, 2006).

2.3.3. Biochemical identification of Campylobacters:

The preliminary identified Campylobacter species were further subjected to catalase test, oxidase test, susceptibility to nalidixic acid and cephalothin and rapid hippurate hydrolysis test (Nachamkin, 1999).

2.4. Molecular identification of isolates:

2.4.1. DNA extraction:

DNA extraction from the biochemically identified isolates was performed according to the manufacturer guidelines using Bacterial DNA Extraction Kit (Spin-column) (BioTeke Corporation, China).

2.4.2. Confirmation of Campylobacter spp. by PCR:

A conventional PCR targeting 650 bp of 23S rRNA specific for Campylobacter spp. was used for the confirmation of 42 biochemically identified campylobacter isolates (Wang et al., 2002). The sequences of primers are Camp-F 5'-TATACCGGTAAGGAGTGCTGGAG-3' and Camp-R 5'- ATCAATTAACC TTCGAGCACCG-3'.

2.4.3. Confirmation of C. jejuni and C. coli isolates by real time PCR (rtPCR):

A real time probe based PCR (rtPCR) reaction was used for the confirmation of 28 biochemically identified C. jejuni isolates using species-specific primer and TaqMan probe sets targeting hipO gene specific for C. jejuni (LaGier et al., 2004). The sequences of primers and probe are Cj-F 5'-TGCTAGTGAGGTTGCAAAAGAATT-3', Cj-R 5'-TCATTTCGCAAAAAAA TCCAAA-3' and Cj-FAM 5'-ACGATGATTAAATTCACAATTTTTTTC GCC AAA-3'. Also, 14 suspected C. coli isolates were confirmed by the amplification of glyA gene (LaGier et al., 2004). The sequences of primers and probe are Cc-F 5'-CATATTGTAAAACCAAAGCTTATCGG-3', Cc-R 5'- AGTCCAGCAAT GTGTGCAATG-3' and Cc-VIC 5'-TAAGCTCCAACTTCATCCGCAATCTCT C TAAATTT-3'. Non-template DNA and positive controls of C. jejuni, C. coli, E. coli, S. Typhimurium, Staph. aureus and two

biochemically identified Campylobacter isolates other than C. jejuni and C. coli were also run to determine the specificity of the reaction.

2.5. Antimicrobial susceptibility testing (determination of phenotypic resistance):

2.5.1 Disk diffusion method (Qualitative susceptibility testing):

A total of 28 *C. jejuni* and 14 *C. coli* isolates were examined for their susceptibility to macrolides by the disk diffusion method on Müeller-Hinton agar supplemented with 5% of lysed horse blood 50% following the NCCLS recommendations (CLSI, 2012). *Campylobacter jejuni* NCTC 11322 / ATCC®29428 was used as a quality control. The used macrolides antibiotics (Oxoid) were erythromycin (E_{15} µg), clarithromycin (CLR_{15} µg), azithromycin (AZM_{15} µg) and Spiramycin (S_{100} µg). Tylosin (TLS_5) was not commercially available in the form of discs and prepared from powder (Sigma Aldrich) using Whatman filter paper no. 1.

2.5.2 Broth microdilution method (quantitative susceptibility testing):

Two-fold broth microdilution method was used to determine the minimum inhibitory concentrations (MIC) of 28 *C. jejuni* and 14 *C. coli* strains against macrolides agents using Müeller Hinton broth according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2012).

2.5.3 Interpretation of antimicrobial susceptibility tests

The interpretation criteria for the susceptibility testing of *Campylobacter* spp. for erythromycin, clarithromycin, tylosin and azithromycin were according to EUCAST (2017). The interpretation criteria for tylosin were used the breakpoints given for erythromycin. The criteria for spiramycin and tylosin were following the recommendations of Ca-SFM (2013).

3. RESULTS

3.1. Preliminary confirmation of thermophilic Campylobacter species

Campylobacter species were preliminary identified by their colonial morphology on mCCDA and sheep blood agar. The colonies appeared greyish, flat, moistened, with a tendency to spread and they may have a metal sheen suggesting *C. jejuni* isolates. Creamy-grey moist and more discrete colonies suggested that the colonies belong to *C. coli*. Additionally, on 5-7% lysed horse blood agar *Campylobacter* spp. had characteristic colonies of oil drop like appearance (translucent droplet-like colonies), slightly pink, round, convex, smooth and shiny, with regular edges. Occasionally, *Campylobacter* spp. showed greyish, flat, moistened, with a tendency to spread on lysed horse blood agar.

Campylobacter species were also confirmed by production of oxidase, the results were indicated by intense deep purple color appearance within few seconds on oxidase strip. The suspected Campylobacter organisms in freshly prepared cultures appeared as Gram negative (faint in color) curved bacilli. In old cultures, or when exposed to air for prolonged time periods, colonies transformed from spiral form to coccoid morphology.

Examination of motility under oil immersion lens showed that campylobacters are highly motile with characteristic corkscrew like motility, while in old cultures they were less motile. Moreover,

thermophilic campylobacters did not grow at 25°C in a microaerobic atmosphere or at 41.5°C aerobically for 48 hours.

3.2. Identification of Campylobacter species:

The results showed that all examined isolates (n=167) were positive for catalase production. All campylobacter isolates (100%) were resistant to nalidixic acid, therefore, it was difficult to differentiate *C. lari* and *C. coli*, while, *C. jejuni* was differentiated by rapid Sodium hippurate hydrolysis test. The results of Sodium hippurate hydrolysis test revealed that 95 out of 167 (56.9%) of isolates were positive, classifying them as *C. jejuni*.

3.3. Occurrence of Campylobacter spp. in different samples:

According to the phenotypic identification; *Campylobacter* spp. were isolated from 29.4% of the examined samples. The results demonstrated a high isolation rate of *Campylobacter* spp. in chickens from caecal part (41.2%), followed by thigh meat (31.4%), cloacal swabs (29.3%) and breast meat (27.5%).

Identification of campylobacters to the species level showed that *C. jejuni*, *C. coli*/*C. lari* and *C. hyointestinalis* were identified in 56.9, 40.1 and 3% of the examined samples, respectively. The highest isolation rate of *C. jejuni* was detected in caecal parts (80.9%), followed by cloacal swabs (55.3%), neck skin (53.8%) and breast meat (50%). The lowest isolation rate was in thigh meat (43.7%), (Table 1).

3.4. Molecular confirmation of representative campylobacter isolates:

3.4.1. Confirmation of campylobacter isolates by conventional PCR:

Forty-two campylobacter isolates (28 and 14 biochemically suspected *C. jejuni* and *C. coli* isolates, respectively) were confirmed by the amplification of 23S rRNA gene; an amplicon of 650 bp size was generated using conventional PCR.

3.4.2. Confirmation of C. jejuni and C. coli isolates by rtPCR:

Real time PCR targeting *hip*O gene specific for *C. jejuni* and *gly*A specific for *C. coli* were used for the confirmation of the selected phenotypically identified isolates. The results showed that all 28 *C. jejuni* and 14 *C. coli* isolates were confirmed by rtPCR. The specificity of the reactions was confirmed when primers and probes targeting *hip*O and *gly*A genes did not amplify DNA from other controls.

3.5. Antimicrobial susceptibility testing:

The results obtained from both methods revealed that all *C. jejuni* and *C. coli* isolates were resistant to antimicrobials of macrolides class. To overcome the difficulty in reading MIC results due to the presence of LHB, resazurin was used and recording of the results was based on the observation of color change. Active viable bacterial cells reduce the reagent (purple – blue) to pink colorless. The MIC after adding resazurin indicator was defined as the lowest antibiotic concentration that prevented the reagent color change (Figures: 1). The results of broth microdilution method revealed that *C. jejuni* and *C. coli* isolates were 100% resistant to antibiotics of FQ and macrolides classes.

Table 1: Occurrence of different *Campylobacter* spp. in the examined samples

Type of samples	Number examined	Number (proportion %)			
		Total campylobacter isolates*	*C. jejuni***	*C. coli* / *C. lari***	*C. hyointestinalis***
Cloacal swabs	364	103 (28.3%)	57 (55.3%)	42 (40.8%)	4 (3.9%)
Neck skin	51	13 (25.5%)	7 (53.8%)	6 (46.2%)	0 (0%)
Breast meat	51	14 (27.5%)	7 (50%)	7 (50%)	0 (0%)
Thigh meat	51	16 (31.4%)	7 (43.7%)	9 (56.3%)	0 (0%)
Caecal parts	51	21 (41.2%)	17 (80.9%)	3 (14.3%)	1 (4.8%)
Total	568	167 (29.4%)	95 (56.9%)	67 (40.1%)	5 (3%)

* The isolation rate was calculated in relation to the total number of the examined samples.

** The isolation rate of each species was calculated in relation to the total no. of the isolated campylobacters.

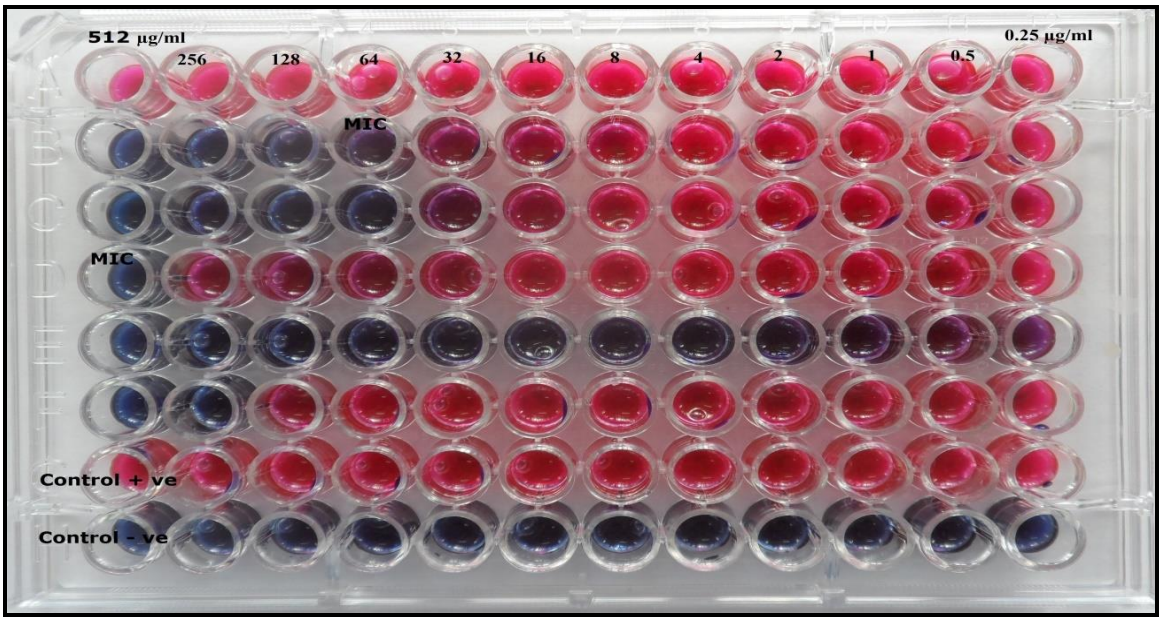

Figure 1: Reading results of broth microdilution test using resazurin reagent. Positive results; pink color, Negative results; blue or purple color, MIC; the lowest antibiotic concentration that prevented the reagent color change (blue color).

4. DISCUSSION

For the identification of thermophilic campylobacters to the species level, catalase test, susceptibility to nalidixic acid and cephalothin and rapid hippurate hydrolysis test were performed. In the current study, the results showed that all campylobacter isolates were resistant to nalidixic acid. As a result, differentiation between *C. jejuni*, *C. lari* and *C. coli* based on the susceptibility to nalidixic acid was difficult.

The judgment on hippurate hydrolysis test which differentiates *C. jejuni* from *C. coli* isolates, is usually based on qualitative criteria which are not reliable and may lead to misinterpretation (Megraud, 1987). Thus, isolates which were used for further investigations for the detection of FQ and macrolides resistance were confirmed by PCR.

In the current study, *Campylobacter* spp. were isolated from 29.3% cloacal swabs. Similar isolation rate of 29% from broiler flocks was reported in Japan (Sabike et al., 2017). Nearly similar results of 35.1% (Abd El-Tawab et al., 2015) and 21.6% (Awadallah et al., 2014) were reported in Egypt. Comparable isolation rates of 31.9% in Vietnam (Carrique-Mas et al., 2014) and 39.2% in Estonia (Mäesaar et al., 2014), were also reported.

Different studies reported higher prevalence rates of *Campylobacter* spp., for instance; 58% in Brazil (Da Silva et al., 2016), 85% in Algeria (Messad et al., 2014) and 57% in NewZeland (Anderson et al., 2012). The aforementioned higher isolation rates could be attributed to the isolation of *Campylobacter* spp. from fresh fecal samples on the ground. The farm ground near poultry houses are suspected to be highly contaminated with *Campylobacter* spp. from different sources such as wild birds, rodents and free living pets (Studer et al., 1999). In addition, Pezzotti et al. (2003) and Salihu et al. (2012) reported higher isolation rates of 82.9% in Italy and 51.5% in Nigeria from chicken cloacal swabs, respectively. Such higher percentages compared to the obtained results during the current study could be a result of campylobacter identification by only conventional methods in the two studies reported in Nigeria and Italy. However, an isolation rate of 69.8% from chicken cloacal swabs determined by PCR in Tanzania was reported (Mdegela et al., 2006). The authors attributed such high percentage to the extensive type of chicken management that increases the exposure of birds to campylobacter infection through insects, rodents, contaminated water and poor housing hygiene (Mdegela et al., 2006).

Lower *Campylobacter* spp. isolation rates of 1.5% and 6.9% from cloacal swabs were reported in Greece (Marinou et al., 2012) and Italy (Menna et al., 2005). The authors attributed the low isolation rate to strict biosecurity measures observed in the examined farms.

Out of the 103 campylobacter isolates from cloacal swabs, 55.3% were identified as *C. jejuni*. Comparable percentages of 46% in Egypt (Abd El-Tawab et al., 2015) and 68.1% in Argentina (Zbrun et al., 2015), were reported.

Higher isolation rates of *C. jejuni* were reported in different studies; 90% in Great Britain (Jorgensen et al., 2011) and 89% in Vietnam (Carrique-Mas et al., 2014). However, lower prevalence rate of 31.4% was obtained in Reunion Island (Henry et al., 2011). In Greece, *C. coli* was only identified from chicken cloacal swabs (Marinou et al., 2012). The authors attributed such results to the type of feed ration because *C. jejuni* do not frequently colonize in birds receiving plant protein based feed (Udayamputhoor et al., 2003).

Poultry is exposed to campylobacters at farm level due to insufficient biosecurity measures at the market outlets due to contamination of carcasses during different slaughtering processes (Parkar et al., 2013). *Campylobacter* spp. were isolated from 27.5 and 31.4% of the examined breast and thigh meat samples, respectively. Comparable percentages of campylobacters were reported in chicken meat; 34.1% in Japan (Stella et al., 2017), 21.7% in Ethiopia (Dadi and Asrat, 2016) and 29.1% in Greece (Economou et al., 2015).

Higher isolation rates of campylobacters were documented; 61.6% in Italy (Pedonese et al., 2017), 63.1% in China (Zhu et al., 2016) and 58.8% in Korea (Wei et al., 2016). While lower isolation rates of 17% in Brazil (Da Silva et al., 2016) and 17.2% in China (Zhang et al., 2016), were also reported.

The variation in *Campylobacter* spp. isolation rates among the previously mentioned studies could be attributed to difference in the sanitation levels during handling and processing of chicken, season of sampling and the laboratory methodologies employed for isolation (Shih, 2000).

C. jejuni was isolated from 43.7 and 50% of thigh and breast meat samples, respectively. Comparable percentage of *C. jejuni* isolation from chicken meat were documented in several studies; for instance, 41.9% in Italy (Pedonese et al., 2017) and 52.5% in Korea (Wei et al., 2016). Higher isolation rates of 76.9% in China (Zhang et al., 2016) and 88.8% in Estonia (Mäesaar et al., 2015), were reported.

The obtained results in the current study were lower than those reported in Japan where *C. jejuni* isolates were identified from 86.2 and 78.6% of breast meat and thigh meat samples, respectively (Sallam, 2007). However, in Egypt, Saad (2014) reported the identification of *C. jejuni* from 6.9% of the examined thigh meat samples.

Chicken skin provides suitable microenvironment for the survival of campylobacters due to accumulation of water which increases the surface area available for bacterial contamination (Miwa et al., 2003). The isolation rate of campylobacters from skin samples was 25.5%, of which, 53.8% were identified as *C. jejuni*.

Different studies also reported the isolation of *Campylobacter* spp. from chicken skin samples; 68% in Sweden (Hansson et al., 2015), 30.8% in Egypt (Abd El-Tawab et al., 2015) and 68% in Sweden (Hansson et al., 2015).

The detection of campylobacter in carcass skin varied significantly due to the fact that the slaughtering process varies between different slaughter houses and the degree of external contamination of the feathers during transport to slaughter can vary (Hansson et al., 2015).

Manual slaughtering and evisceration, which is common in poultry pluck-shops based markets in Egypt, where fecal content leakage is common, may result in contaminating chicken meat (breast and thigh meat) and skin. The same observation was reported by Huang et al. (2016) in China and Saad (2014) in Egypt. The authors

concluded that chicken from wet markets that devoid hygienic measures were frequently and heavily contaminated with campylobacter.

Campylobacter spp. are ubiquitous foodborne pathogens that colonize the intestinal tract of chicken especially the caeca (Silva et al., 2011). *Campylobacter* spp. isolation rate of 41.2% from chicken caecal parts was obtained in the current study, of which, 80.9% were identified as *C. jejuni*. Comparable results were also reported as 56.1% in China (Han et al., 2016) and 41% in Egypt (Abd El-Tawab et al., 2015).

Higher isolation rates were previously obtained; 100% in Sweden (Hansson et al., 2015) and 98% in Algiers (Messad et al., 2014).

Gblossi Bernadette et al. (2012) reported that *Campylobacter* spp. are better detected by direct examination of the intestine than in the case of cloacal swabs. Such assumption was based on the fact that cecum is the main colonization site of *Campylobacter* spp. in chicken (Silva et al., 2011). The aforementioned higher isolation rates could be attributed to the isolation of campylobacters from caecal contents, where the load of bacteria could reach 10^{10} organisms per gram of caecal content (Silva et al., 2011).

The variation in *Campylobacter* spp. isolation rate between different studies could be attributed to different possible reasons, such as the type and site of the examined samples (Meremäe et al., 2010). Moreover, the seasonal factors, biosecurity, husbandry and management and production system have the greatest impact on the prevalence rate of campylobacters (Chatur et al., 2014; Newell et al., 2011).

Another factor that might have an influence on the isolation rate of *Campylobacter* spp. is the age of the examined chickens (Newell et al., 2011). The contamination of broiler flocks by campylobacters generally occurrs late (after 15–20 days of rearing) due to ability of the gut flora of the young birds to provide good protection against campylobacter colonization (Laisney et al., 2004). The prevalence of *Campylobacter* spp. in chicken is expected to be high in broilers slaughtered at 5-6 weeks (Bouwknegt et al., 2004), while in older chickens, the prevalence decreases reflecting acquired immunity (Kalupahana et al., 2013). During the current study, the examined samples were collected from chickens at 6 weeks old explaining the relatively high isolation rate of campylobacters.

Moreover, the methodology; isolation and identification techniques, has an impact factor which affected the analytical results (Mead et al., 2010). For example, Salvat et al. (2017) noticed highly significant decrease of heavily campylobacter contaminated carcasses when sampling method changed (from neck to leg skin) in a traditional free range broiler production along 23 year survey program. The isolation methodologies are laborious, and there are many broths and agars available. Some studies have evaluated the effectiveness of different broths and agar plates for their ability to isolate campylobacter from several

matrices to develop more efficient and lower cost methods (Gonsalves et al., 2016). Seliwiorstow et al. (2016) demonstrated the impact of culture medium on the recovery of campylobacters from fresh and frozen naturally contaminated poultry meat samples with a great effect on the detection of campylobacters. Also, Oyarzabal et al. (2005) concluded that certain agars and broths are better than others for the isolation of campylobacter form certain samples with regards to time, preparation, performance and cost.

Although all that mentioned possible reasons, there is need for further research to explain the possible reasons on *Campylobacter* spp. distribution differences among studied company farms (Huang et al., 2016).

The results obtained from both disc diffusion and broth microdilution testing methods in the current study showed that all *C. jejuni* and *C. coli* isolates (no=42) were 100% resistant to all examined macrolide agents. In accordance, the same findings were reported in Egypt (Hefny, 2014; Saad, 2014).

The uncontrolled and misuse of tylosin and erythromycin in poultry production in Egypt could be the reason for the high level of macrolides resistance in the current study. Ladely et al. (2007) and Lin et al. (2007) reported significantly increased frequencies of macrolides resistance when tylosin was administered at sub-therapeutic levels in *Campylobacter* species. Also, erythromycin resistance rate among *C. jejuni* and *C. coli* isolates increased after tylosin administration (Ladely et al., 2007). This conclusion was supported by Bester and Essack (2012) in South Africa, who reported that 88% of campylobacter isolates from poultry raised commercially; using macrolides agents, were macrolides resistant versus 0% for those isolates from small-scale family farms where no macrolide agents were used.

Unfortunately, resistance rates are much higher in parts of Asia and Africa; for example, in Nigeria, nearly 80% of strains are macrolides-resistant (Smith et al., 1999). In the contrary, 100% sensitivity of *C. jejuni* isolates was reported in South Korea (Oh et al., 2017) and Spain (Pérez-Boto et al., 2015), where the use of macrolides was restricted.

In conclusion, the relatively high isolation rate of campylobacters from different parts of chicken carcasses during the current study could be attributed to lack of hygienic measures in pluck-shop markets. Thus, the control of campylobacter incidence in poultry is a major public health strategy for prevention of human campylobacteriosis. Also, the high levels of macrolide resistance in *C. jejuni* and *C. coli* isolates were reported which could be attributed to the widespread use of macrolides in chicken production. The current study recommends further studies on the mechanisms macrolides resistance in campylobacters.

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