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Original Article

Detection of Campylobacter in Chicken Parts by Conventional Methods and Polymerase Chain Reaction with Identification of Antibiotic Resistance Profile

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Abstract

Background & Objective(s): Campylobacteriosis is a zoonotic, food-borne bacterial disease caused by Campylobacter spp. The most common pathogenic species are Campylobacter jejuni (C. jejuni) and C. coli. Multiple reservoirs harbor Campylobacter but chicken are considered the most common. Different chicken parts can harbour Campylobacter, particularly the intestine while chicken breasts usually have minimal counts. Antibiotics are used as feed as well as for therapeutic purposes in animals, and thus antimicrobial resistance of some Campylobacter isolates to common antibiotics is an issue of public health importance. The aim of this study was to detect C. jejuni and C. coli in chicken using conventional methods (culture followed by biochemical tests) and PCR, with identification of antimicrobial resistance of isolates.

Methods: In the present study, Campylobacter was isolated from 100 different chicken parts (thigh, neck, intestine and wings) collected from 40 different chickens. Culture on charcoal cefoperazone deoxycholate agar (CCDA) was followed by biochemical confirmation of Campylobacter spp then by matrix-associated laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). Simultaneously, DNA of Campylobacter was detected from chicken broth by multiplex polymerase chain reaction (PCR). Both conventional and PCR methods were compared. Campylobacter colony count was determined for different chicken parts, and the antimicrobial resistance of isolates was identified.

Results: Out of the 100 examined chicken samples, 79 were presumptively positive on CCDA while only 15 isolates were MALDI-TOF confirmed (18.98%). All samples had Campylobacter counts exceeding 104 cfu/g. Colony counts ≥105 cfu/gm were encountered in 77.7% of PCR positive samples. Multiplex PCR had low sensitivity (60%) for detection of Campylobacter in chicken broth compared to confirmed cultures. Despite this drawback, PCR was advantageous over culture in detecting samples with mixed Campylobacter species. The intestine had the highest frequency (27.5%) of Campylobacter, with 72.7% of its samples yielding ≥105 cfu/g. C. jejuni responded better to erythromycin, ciprofloxacin and chloramphenicol (susceptibility= 100%, 80% and 80% respectively) while C. coli had a poorer susceptibility profile. Tetracycline and nalidixic acid had a poor antibacterial effect on both C. jejuni and C. coli.

Conclusion: The distribution of Campylobacter species varied according to chicken part, with the intestine having the highest counts. All chicken samples had Campylobacter counts more than 10 4 cfu/g. PCR had 60% sensitivity compared to culture, but was more superior in detecting mixed cultures. C. jejuni was more sensitive to erythromycin, ciprofloxacin and chloramphenicol antibiotics than C. coli.

Keywords: Campylobacter; chicken parts; CCDA; multiplex polymerase chain reaction (PCR); matrix-associated laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)

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INTRODUCTION

Poultry is the main source of animal protein for many developing countries. It contributes to 45 percent of Egyptian total animal protein consumption.(1) Improperly cooked chicken can act as a vehicle for the transmission of foodborne bacteria, such as Salmonella spp, Enterohaemorrhagic Escherichia coli and Campylobacter spp.(2) In Egypt, El-Tawab et al., (2015) and Omara et al., (2015) reported the occurrence of

Campylopbacter in raw chicken to be 6.2% and 40% respectively.[3, 4]

Campylobacter spp occurs as normal bacterial flora in the intestinal tract of poultry; since the avian temperature is suitable for the growth of those thermophilic organisms (optimum temperature for Campylobacter is 42 °C). After slaughtering, Campylobacter may translocate from the intestine to poultry parts and products.[5] Almost 90% of infections are caused by Campylobacter jejuni (C. jejuni) and Campylobacter coli (C. coli). The main presentation is watery diarrhea, mediated through the cytolethal distending toxin (CDT). Possible complications of campylobacteriosis include Guillain–Barre syndrome (an acute peripheral demyelinating polyneuropathy that lasts for several weeks), reactive arthritis, meningitis and myocarditis in infants. Moreover, in immune com promised patients, Campylobacter might cause a fatal systemic infection.

Campylobacter are gram-negative, spiral, flagellated bacilli with a corkscrew-like motion, except for C.gracilis which is non-motile.[6, 7] Campylobacter is conventionally cultured on charcoal cefoperazone deoxycholate agar (CCDA) in microaerobic conditions. Despite being known as a selective medium, other species may grow on CCDA hindering its selective ability. Other methods include culture on chromogenic media as well as PCR targeting specific genes. The latter method is advantageous due to its ability to detect viable non-culturable (VBNC) forms of Campylobacter that may go undetected by culture.[3, 6]

Fluoroquinolones and macrolides are the drugs of choice for cases requiring treatment. Campylobacter resistance to these two classes of antibiotics is a major concern for public health. The pattern of resistance among C. jejuni and C. coli is highly variable with respect to the country of isolation. Differences may well be related to the source of Campylobacter isolates and the frequency and type of antimicrobial agents used as feed additives or for treating animal and human infections in different geographical areas.[8]

The aim of this study was to detect C. jejuni and C. coli in chicken parts using conventional methods (culture followed by biochemical tests) and PCR, with identification of antimicrobial resistance of isolates.

METHODS

The present cross sectional study was carried out over a period of 14 months, from October 2016 to December 2017. Based on a previous study with an incidence rate of 6.2% of *Campylobacter* spp. in food,[4] using a power of 80% to detect the frequency, precision of 5%, α=0.05, the minimal required sample size was found to be 90. Sample size was increased to 100 to control for attrition bias. The sample size was calculated according to Charan and Biswas.[9]

Sample collection:

One hundred fresh raw samples of chicken parts were collected (thighs n= 20, wings n= 20, necks n= 20, intestine n=40) of 40 chickens. Samples were collected from different poultry markets in Alexandria over a period of 15 months. The markets were selected to represent different districts in Alexandria. Samples were delivered to the laboratory within 2 hours in an icebox and analysis was carried out immediately.

Sample processing and laboratory procedures:

Ten grams of each sample were homogenized in 90 mL of 1% buffered peptone water. This broth was incubated then used for culture and PCR. Regarding culture, serial 10-fold dilutions were done until reaching a dilution of 10^{-5}. A direct plating method was performed, where 100 µL from the previous dilutions were spread on the surface of CCDA agar plates with antimicrobial supplements (SR0155E, Oxoid, United Kingdom) and then incubated under microaerophilic conditions at 42°C for 48 h.[10] Excess moisture during microaerobic incubation can lead to undesirable confluent or swarming growth of *Campylobacter* owing to its high motility. Accordingly, excess moisture was avoided by the addition of 4 – 5 drops of glycerol onto a piece of filter paper in an uncovered petri dish along with the plates in the chamber. The total number of colony forming units (CFU) was determined from countable plates (30 to 300 CFU). The bacterial counts were then multiplied by the dilution factor.[3, 10, 11, 12] An additional volume of broth (10^{-1} dilution) was frozen at -20 °C for further use in PCR.

Identification of *Campylobacter* isolates:

Isolates that were grey in colour, moist, glossy, flat spreading colonies with or without a metallic sheen were presumptively identified as *Campylobacter*. Such colonies were further cultured on Columbia blood agar and microaerophilically incubated at 42 °C for 48 h.[12] Microscopic examination and biochemical tests were done to identify isolates according to the conventional microbiological methods in the ISO protocol .[13] Isolates which were oxidase positive were then sub-cultured on triple sugar iron (TSI) agar. *Campylobacter* on TSI agar showed either no change or alkaline/ no change. Isolates were also subjected to indole, methyl red, Voges-Proskauer, citrate, urease and catalase tests to confirm the identification of *Campylobacter*.[11, 13] All reactions were recorded after microaerophilic incubation at 42°C. Colonies which were biochemically positive were confirmed by Matrix-associated laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Autoflex, Bruker Daltonics, Germany) by the direct application method.[12]

Antimicrobial resistance testing:

All confirmed isolates of *C. jejuni* and *C. coli* were subjected to antimicrobial resistance testing using the following 5 antimicrobial agents: erythromycin (E) (15µg),

tetracycline (TE) (30μg), chloramphenicol (C) (30μg), ciprofloxacin (CIP: 5 μg), and nalidixic acid (NA) (30 μg).[8, 13] Disc diffusion was carried out on Mueller-Hinton agar plates supplemented with 5% defibrinated horse blood. Inhibition zones were measured, recorded and interpreted as susceptible(S) intermediate (I) resistant (R).[13, 14]

PCR for *Campylobacter*

Polymerase Chain Reaction (PCR) was used to detect the presence of 16SrRNA, ask and cj0414 genes of Campylobacter in chicken broth samples. The 16SrRNAgene targeted the genus Campylobacter, while ask gene encoded C. coli and the cj0414 gene was used to detect C. jejuni. DNA extraction was done for Campylobacter using proteinase K as described by Šabatková et al.[15] The integrity of the extracted genomic DNA was assessed by agarose gel electrophoresis. The amplification reaction was performed using multiplex PCR technique. Briefly, primers for 16SrRNA ask and cj0414 amplification were purchased lyophilized (Sigma-Aldrich Co Ltd, Germany). They were reconstituted with appropriate volumes of sterile distilled water and were further diluted (1:10) and the contents were divided into aliquots and stored frozen at -20°C (Table 1). The extracted DNA was subjected to PCR using 2X PCR master solutions I-TaqTMmix (Thermo Scientific, Waltham, United States) a ready- to- use solution.(18)

Reaction mixtures were prepared using sterile nuclease tubes. To each tube a total volume of 25 µl was reached by adding 12.5µl of Taq Green PCR Master mix (2X), 1.0 µl of each of the forward and reverse primers of 16S rRNA, C. jejuni and C. coli. In addition, 5 µl of DNA template (sample) and 0.5 µl of nuclease free water were added to the reaction mixture. For negative control, 10 µl nuclease – free water were used instead of the sample. For positive control, 10 µl of known Campylobacter DNA positive were used. The tubes were transferred to the thermo cycler (Boeco, Germany), and subjected to initial denaturation at 95°C for 1 min, then 35 cycles of denaturation (95°C for 30 sec) , annealing (57°C for 30 sec) and extension(72°C for 4 min) . This was followed by a cycle of final extension (72°C for 4 min).[17, 18] PCR products were loaded on 2% agarose in Tris Borate EDTA (TBE) containing 0.5 µlg of ethidium bromide per ml. After electrophoresis, the amplicon was visualized on agarose gel using UV transilluminator. The gel was examined for specific bands of DNA fragments.

Statistical analysis of data:

Data were collected and analyzed using SPSS (Statistical Package for Social Science) software (version 21.0). Data were entered as numerical or categorical, as appropriate. Kolmogorov-Smirnov test of normality revealed no significance in the distribution of the variables, so the parametric statistics was adopted. Categorical variables were described using frequency and percentage. Chi-square test was used to test association between qualitative variables. Monte Carlo corrections were carried out when indicated. Cohen's kappa coefficient (κ) which measures inter-rater agreement for qualitative (categorical) items was used. A modification to Cohen's kappa (weighted Cohen's kappa) was also used. For assessment of kappa coefficient, Landis and Koch magnitude guidelines for agreement was used. Specific agreements (positive, negative agreement and intra-class correlation (ICC) were calculated. Diagnostic test evaluation was carried out using MedCalc Software version 14. An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%.[19, 20, 21]

Table (1): The sequence of primers for Campylobacter genus, C. jejuni and C. coli [16,17]

Primers	Target gene	Specificity	Primer sequence (5`- 3`)	Size of PCR Product (bp)
C412F	16S rRNA	Campylobacter genus	5'- GGATGACACTTTTCGGAGC-3`	816
C1228R	16S rRNA	Campylobacter genus	5'-CATTGTAGCACGTGTGTC-3'	816
CC18F	ask	C. coli	5'-GGTATGATTTCTACAAAGCGAG-3'	502
CC519R	ask	C. coli	5'-ATAAAAGACTATCGTCGCGTG-3'	502
C1	cj0414	C. jejuni	5'-CAAATAAAGTTAGAGGTAGAATGT-3'	161
C3	cj0414	C. jejuni	5'-CCATAAGCACTAGCTAGCTGAT-3'	161

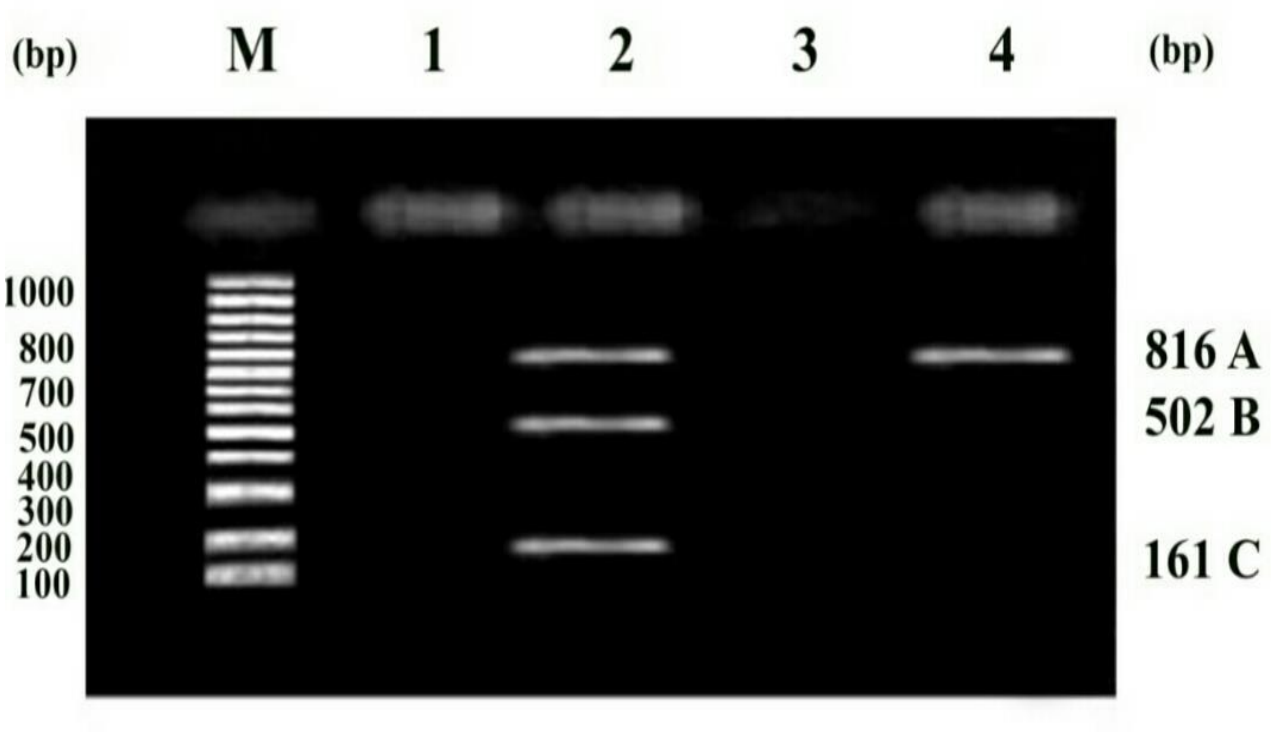

Figure (1): Agarose gel electrophoresis of multiplex PCR for detection of 16SrRNA, C. jejuni and C. coli

A – 16s rRNA
B - C. coli
C - C. jejuni
Lane M- marker 100bp DNA ladder
Lane 1 and 3 show negative samples
Lane 2 shows mixed C.jejuni and C.coli
Lane 4 non -C.jejuni and non C.coli strains

RESULTS

Rate of detection of Campylobacter by culture and PCR

Campylobacter spp were presumptively identified in 79% of samples by culture on CCDA, but only 21 samples (21%) were biochemically proven to be Campylobacter spp. Furthermore, this number decreased to 15 (15%) when MALDI TOF was used as a confirmatory tool following culture. All false positive isolates (6 isolates) were proven by MALDI TOF to be Pseudomonas aeruginosa and Pseudomonas mendocina. Even lesser numbers of Campylobacter spp were detected by PCR from chicken broth, where only 9 samples out of 100 broth samples were positive by PCR (Table 2). Out of the 21 biochemically positive samples, 6 samples were later proven by MALDI-TOF to be Pseudomonas aeruginosa and Pseudomonas mendocina. Out of the 40 intestine samples, Campylobacter spp was confirmed in 11 samples (27.5%). Two samples (10%) were positive for each of necks and wings samples. None of the thigh samples were positive by culture.

Table (2): Detection of Camypylobacter spp. by different identification methods

Diagnostic method	Chicken samples (n=100) No.	%
CCDA culture		
Presumptively identified as *Campylobacter*	79	79.0
Biochemically confirmed*	21	21.0
MALDI TOF confirmed	15	15.0
PCR confirmed	9	9.0

* Out of the 21 biochemically positive samples, 6 samples were later proven by MALDI-TOF to be *Pseudomonas aeruginosa* and *Pseudomonas mendocina*

Table (3): Distribution of the Campylobacter isolates detected by culture according to their species in different chicken parts

Sample	Species (n=15)		Total	Test of significance
	C. coli	*C. jejuni*		
	No. (%)	No. (%)	No. (%)	
Wing (n=2)	2 (100)	0 (0.0)	2 (100)	$X^2_{(df=4)}$=5.45
Thigh (n=0)	0 (0.0)	0 (0.0)	0 (0.0)	
Intestine (n=11)	7 (63.6)	4 (36.4)	11 (100)	$p_{(MC)}$=0.281 NS
Neck (n=2)	0 (0.0)	2 (100)	2 (100)	
Total	**9 (60)**	**6 (40)**	**15 (100)**	

X^2: Pearson Chi-Square
df: degree of freedom
MC: Monte Carlo Correction for p value of Pearson Chi-Square

Campylobacter species in different chicken parts

C. coli constituted 60% of Campylobacter spp isolated using culture, while C. jejuni constituted 40% of all isolates. Out of the 11 intestinal isolates, 7 of them were C. coli (63.6%) and 4 isolates were C. jejuni (36.4%). Both positive samples from the wings were C. coli, while both isolates from the neck were C. jejuni. There was no statistical significance in the distribution of C. *jejuni* and C. coli among different parts of chicken samples (Table 3).

Campylobacter colony counts in different chicken parts

When the CFU/g was counted for the 15 culture-confirmed samples, 8 intestinal samples (72.73%) yielded >105 CFU/g while 104- <105 counts were detected in intestine, neck and wing samples (Table 4).

Table (4): Distribution of colony count of CCDA culture positive samples according to chicken parts

Positive samples	Colony count (CFU/g)	
	(10^{4} - $<10^{5}$)	($\geq 10^{5}$)
Wing (n= 2)	1 (50.0%)	1 (50.0%)
Intestine (n= 11)	3 (27.3%)	8 (72.73%)
Neck (n=2)	1 (50.0%)	1 (50.0%)
Total (n=15)	5 (33.3%)	0 (66.67%)
Test of significance	$X^2_{(df=4)}$=8.925 $p_{(MC)}$=0.053 NS	

X^2: Pearson's Chi square test df: degree of freedom
MC: Monte Carlo correction for p value of Pearson's Chi square
*: statistically significant (p<0.05) NS: Statistically not significant (P< 0.05)

Campylobacter colony counts versus PCR positivity

Overall, the type of chicken part did not significantly affect the colony count of Campylobacter positive samples (*P*=0.053) (Table 5). The nine positive Campylobacter broth samples by PCR were as follows: 5 samples were positive for C. jejuni (55.56%), 2 samples of C. coli (22.22% of positive samples), while 2 samples (22.22%) were mixed C. jejuni and C. coli. Forty percent of isolates (6 isolates) that were positive by culture had negative results for Campylobacter by PCR

CCDA culture and PCR

There was an almost perfect agreement (kappa=0.704, *P*<0.001) between CCDA culture and PCR from broth, as regards the detection of *Campylobacter* spp. The positive agreement between both methods was 75%. The sensitivity of PCR from broth was found to be 60%, while the specificity and positive predictive values of PCR were found to be 100% each (Table 6).

Table (5): Relation between Campylobacter colony count on CCDA and PCR

Colony forming unit (cfu/g)	PCR
	Positive (n=9)
10^{4} - $<10^{5}$	
Number	2
Percentage within PCR	22.2%
$\geq 10^{5}$	
Number	7
Percentage within PCR	77.78%

Out of the 9 PCR positive samples, 77.7% were associated with count $\geq 10^{5}$cfu

Table (6): Agreement between CCDA culture and PCR regarding the identification of Campylobacter spp.

		PCR		
		Negative	**Positive**	**Total**
CCDA culture	**Negative**	85 (85.0%)	0 (0.0%)	85 (85.0%)
	Positive	6 (6.0%)	9 (9.0%)	15 (15.0%)
	Total	91 (91.0%)	9 (9.0%)	100 (100.0%)

Kappa	0.718
Standard error	0.107
***P* value**	0.000*
Weighted kappa	0.718
Standard error	0.107
95% CI	0.509 to 0.928
Intraclass correlation (ICC)[a]	
- **Coefficient**	0.844
- **95% CI**	0.769-0.895
- **p value**	0.000*

[a] Average measures: this ICC is an index for the reliability of the ratings for one, typical, single rater, This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Sensitivity: 60.00%
Specificity: 100.00%
Positive predictive value: 100.00%
Negative predictive value: 90.63%
Overall accuracy: 91.78% (p= 0.038*)
Proportions of specific agreement:
Negative agreement = 2*58 / (2*58 + 0 +6) = 95.08%
Positive agreement = 2*9 / (2*9 + 0 + 6) = 75.00%

Table (7): Antimicrobial resistance profile of C. jejuni and C. coli isolates from chicken parts

	C. coli (n= 10)	**C. jejuni (n= 5)**
Erythromycin (E)		
Resistant	6 (60.0%)	0 (0.0%)
Susceptible	4 (40.0%)	5 (100.0%)
Ciprofloxacin (Cip)		
Resistant	3 (30.0%)	1 (20.0%)
Susceptible	7 (70.0%)	4 (80.0%)
Nalidixic acid (NA)		
Resistant	9 (90.0%)	5 (100.0%)
Susceptible	1 (10.0%)	0 (0.0%)
Tetracycline		
Resistant	7 (70.0%)	3 (60.0%)
Susceptible	3 (30.0%)	2 (40.0%)
Chloramphenicol (C)		
Resistant	6 (60.0%)	1 (20.0%)
Susceptible	4 (40.0%)	4 (80.0%)

Antimicrobial resistance profile

C. jejuni responded better to erythromycin, ciprofloxacin and chloramphenicol (susceptibility= 100%, 80% and 80% respectively). C. coli had a poorer susceptibility profile when compared to C. jejuni, since 70% of C. coli isolates responded to ciprofloxacin while the rest of antibiotics were less effective, ranging between 10% and 40% sensitivity. Tetracycline had unsatisfactory results where only 40% of C. jejuni isolates and 30 % of C. coli isolates were sensitive to it. Nalidixic acid was the poorest antibiotic since none of C. jejuni and only 10% of C. coli were susceptible to it (Table 7).

DISCUSSION

Campylobacter spp was detected in 15% of chicken samples. Much higher results were reported by El-Tawab et al., (Egypt, 2015), Mansouri et al., (2012), and Workman et al., (2005) (76.9%, 75.6% and 79% respectively).[3, 22, 23] In contrast, much lower isolation rates were obtained in
Egypt by both Shawky et al., (2015) and Omara et al., (2015) (3% and 6% respectively).[24, 4]

In the present study, the frequency of Campylobacter spp in chicken parts was as follows: 27.5% of intestine samples, 15% of chicken necks, 10% of wings and 0% of thigh samples. Awadallah et al., (Egypt, 2015), reported lower rates of intestinal Campylobacter (2.7%) , but higher isolation rates from thigh samples (38.5%) which is much higher than the present study (0.0%).[25]

The variation in Campylobacter isolation rate between different studies could be attributed to several factors such as the type of examined samples, country, climate factors, hygienic level and isolation as well as identification methods. Furthermore, the frequency of Campylobacter species in poultry is expected to be high in broilers slaughtered at 35–42 days, while in older chickens, the frequency decreases reflecting acquired immunity. Thus the age of chicken is another factor controlling the frequency of Campylobacter species.[26]

According to the European Food Safety Authority (EFSA, 2011), all slaughtered poultry batches should comply with a Campylobacter permissible level of 103 CFU/gram. In the current study, all samples had a colony count more than 104 CFU/g, which is higher than the recommendations of the EFSA.[27] Currently, there is no standard for the detection of Campylobacter in chicken in the Egyptian microbiological regulations for poultry.
Campylobacter colony counts of >105 were encountered in the broth of 8 intestinal samples (72.73%) while 104-<105 counts were detected in intestine, neck and wing samples (*P*= 0.053). High intestinal counts could be explained by the fact that the intestine is the natural reservoir of Campylobacter. According to Cawthraw et al., (1996) poultry are colonized throughout their gastrointestinal tract; colonization of the caecum can reach 109 CFU\ml per gram of caecal contents.[28] On the contrary to the findings in the present study, Baré et al., (2013) reported that the highest Campylobacter concentrations were detected in the chicken necks (3.45 ± 1.10 CFU/g) and the abdominal skin, while the lowest were found on the breast and the thigh skin (2.96 ± 1.00 CFU/g). Baré et al., (2013) attributed the high neck counts to a baseline higher contamination of the neck skin, or, the gravitational effect on water trickling with bacteria during slaughter line hanging. In their study, the mean concentrations of Campylobacter recovered from the different chicken sites were not significantly different.[29] A European baseline study recommended the analysis of pooled skin samples for a better estimation of the Campylobacter level in chicken carcasses.[27]

The current study showed that C. coli was predominant in wings and intestine (100% and 60% respectively of their isolates). C. jejuni was mainly present in neck samples followed by intestine samples (66.67% and 30% respectively of their isolates). Furthermore, 33.3% of positive neck samples and 10% of positive intestine samples were mixed C. jejuni and C. coli. Nine chicken broth samples were positive by PCR for C. jejuni or C. coli or both. Those samples were as follows: 2 samples of C. coli (22.22% of positive samples), 5 samples were positive for C. jejuni (55.56%), while 2 samples (22.22%) had both species. Jribi et al., (Tunisia, 2017) reported that the most frequently isolated species was C. jejuni (59.7%).(30) In contrast, Malik et al., (2014) found that out of the 32 Campylobacter isolates from chicken samples, 93.5% were C. coli and 6.25% were C. jejuni.[31] In the present study, the positive agreement between culture and PCR was found to be 75% which is similar to results by Lawson et al, (1999) (77.5%).[32] In the present study, the sensitivity and specificity of PCR in relation to the conventional culture method were found to be 60% and 100% respectively. Kulkarni et al, (2002) reported that PCR was more superior to culture (culture detected only 85% of PCR-positive cases) and attributed this to DNA extraction within 24 hours of receipt of the specimens.[33] On the contrary, Lawson et al (1999) performed DNA extraction within 10 days and reported less PCR sensitivity.[32] This delay might have resulted in degradation of Campylobacter DNA and hence lower PCR sensitivity. In this present study, DNA extraction for PCR was done within one week of sample collection, which might have contributed to this low PCR sensitivity, according to the assumption of Kulkarni et al. (2002).[33] Further work would be needed to confirm this hypothesis. Another possible explanation for reduced PCR sensitivity would be the presence of some inhibitory substances in food. A further disadvantage of PCR methods is the lack of an isolate, and hence the inability to perform antibiotic sensitivity testing or typing for epidemiological purposes. Moreover, its cost renders its use in routine use not cost-effective.[32, 33] In the present study, mixed infections with C. jejuni and C. coli were detected by PCR in 2/ 9 samples (22.22%). Lawson et al, detected mixed infections in

4.60% of their isolates.[32] This ability of PCR to detect mixed Campylobacter species is advantageous over culture, since the cultural morphological similarity of species might cause them to be overlooked during selection of colonies for confirmation. This drawback was observed in our study.

In the present study, six isolates were misidentified by biochemical methods as Campylobacter, and were then proven by MALDI-TOF to be Pseudomonas aeruginosa and Pseudomonas mendocina. Colony morphology and biochemical reactions may lead to false positive results since many reactions give variable undetermined results. Moreover, the hippurate hydrolysis test which is widely used to distinguish C. jejuni (responds positively) from C. coli and C. lari has proven to be unreliable as a sole diagnostic test. This is due to the discovery of C. jejuni subspecies jejuni strains which are negative to the hippurate test.[18] These factors highlight the importance of confirmatory tools (such as MALDI- TOF) other than biochemical methods.[12]

Antibiotics not only play a pivotal role in the treatment and prevention of human and veterinary infections, they are also used in animal feed as growth promoters. The excessive consumption of antibiotics has resulted in increased antibiotic resistance.[34, 35]

In the present study, C. jejuni responded better to erythromycin, ciprofloxacin and chloramphenicol (susceptibility= 100%, 80% and 80% respectively). C. coli had a poorer susceptibility profile when compared to C. jejuni, since 70% of C. coli isolates responded to ciprofloxacin while the rest of antibiotics were less effective, ranging between 10% and 40% sensitivity.
Siddiqui et al.,(2015) reported that 60% of C. coli isolates were resistant to erythromycin, while none of C. jejuni isolates showed resistance.[34] In contrast, Karikari et al., (2017) found higher resistance to erythromycin (96% of C. jejuni strains and 92% of C. coli).[35]

Nalidixic acid was the poorest antibiotic since none of C. jejuni and only 10% of C. coli were susceptible to it. This is in agreement with Girgis et al., where all isolates of C. coli and C. jejuni were resistant to this antibiotic.[36] On the contrary, no resistance was observed among C. coli strains to nalidixic acid as reported by Karikari et al., (2017 and only 33.3% of C. jejuni isolates were resistant.[35]
Tetracycline had unsatisfactory results, where only 40% of C. jejuni isolates and 30 % of C. coli isolates were sensitive to it. In accordance to that, Karikari et al., (2017) found that the resistance to tetracycline was 93.3% in isolates of C. jejuni and 92% of C. coli isolates.[35] It is therefore not recommended to include tetracycline in the animal feed for prophylaxis against Campylobacter due to high resistance to this antibiotic. However, a larger sample size should be included in further studies to confirm this notion.

Overall, the present study recommends further studies on different multiplex PCR protocols for the detection of Campylobacter contamination in chicken parts in order to improve sensitivity. PCR cannot be relied only as a sole diagnostic tool and should be combined with culture. Species identification may be helpful in antibiotic choices in cases requiring therapy. Proper antibiotic administration to poultry should be based on the antibiotic susceptibility pattern to avoid emergence of resistance. The inclusion of Campylobacter in the Egyptian microbiological standards for poultry is highly recommended, coupled with more stringent monitoring for Campylobacter in chicken sold at markets.

CONCLUSION & RECOMMENDATIONS

The proportion of CHC patients who survived seronegative was 0.250 at the 48th week of treatment. Factors associated with seroconversion were age (below 55 years) and HC virus load (< 900,000 IU/ml). CHC patients of 55 years and above, with chronic diseases and those with a HC viral load of ≥ 900,000 IU/ml were found to be statistically associated with poor HRQOL in the domains of physical functioning, role limitations due to physical health problems, role limitations due to emotional problems and pain in comparison with other age groups.
Retired CHC patients had the worst HRQOL on physical functioning only. All the domains of the SF36 decreased by end of the 4th week and started to increase at the 24th and the 48th weeks of follow up period. The same picture was observed in the selected viral HC quality of life questionnaire score, physical health and mental health components.

Identifying the predictors of response to PEG-INF/RBV therapy and tailoring treatment regimens for individual patients based on their risk profile factors may be one approach for achieving maximum antiviral response. These findings will be a baseline for Sovaldi treatment and need to be supported by large, prospective clinical studies that are designed to evaluate the virologic response rates of Sovaldi.

Development of epidemiological models to understand the transmission dynamics of HCV in Egypt, new infections and possibility of eradication of the disease should be carried out. Although HCV eradication is potentially feasible, there remain many barriers that need to be overcome in Egypt. Such barriers include the development of simplified and highly effective drug regimens, the needs assessment for HCV treatment, improving the rates of detection of infection, and the availability of financial resources.

Evaluation of the HRQOL of CHC patients especially those at risk for poor treatment response; older age, those with chronic diseases and those with high HC virus load is important. The questionnaire should be applied through treatment sessions to assess the treatment response and its relation with the quality of life.

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