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Research Article
Multilocus Sequence Typing (MLST) of *Campylobacter jejuni* Isolated From Broiler Meat in Egypt

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Abstract

Background and Objectives: Infection with *Campylobacter jejuni* is one of the most common causes of bacterial gastroenteritis. Infections are mostly acquired due to consumption of raw or undercooked poultry. The aim of this pilot study is to determine the prevalence and the sequence types (STs) distribution of *C. jejuni* isolated from broiler meat in Egypt. **Materials and Methods:** A total of 190 broiler meat samples were collected from retail chicken shops located at Mansoura, Egypt and examined bacteriologically for the presence of *Campylobacter* spp. The biochemically identified *Campylobacter* isolates were confirmed by Multiplex PCR (m-PCR). In addition, multilocus sequencing typing (MLST) was used for genotyping of *C. jejuni* isolates. **Results:** Thirty two *Campylobacter* isolates divided into *C. coli* (25 isolates) and *C. jejuni* (7 isolates) were recovered. Multiplex PCR results found to be 100% in line with biochemical identification. Out of 7 *C. jejuni* isolates genotyped by MLST, 4 isolates were assigned to ST21, 2 isolates were assigned to ST48 and one isolate was assigned to ST464. **Conclusion:** This study provides valuable information concerning the prevalence of thermophilic *Campylobacter* spp. and sequence types distribution of *C. jejuni* recovered from broiler meat for the first time in Egypt. The identified sequence types from this study were frequently reported in human illnesses. Thus, the present results highlight the importance of the retail broiler meat as a significant source for human *Campylobacter* infection.

Key words: *Campylobacter*, broilers meat, multiplex PCR, MLST, sequence type

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Campylobacter has been documented as one of the significant bacterial agents causing food-borne disease worldwide[1]. Among the 25 species and 8 sub-species of *Campylobacter* identified[2], *C. jejuni* subsp. *jejuni* or *C. coli* are associated with more than one host and have zoonotic potential in avian species. Recently, the incidence of human gastrointestinal due to *C. jejuni* infection has been increasing[3]. The previous studies confirmed that *Campylobacter* infection is still endemic in Africa, Asia and Middle East, although epidemiological data from these areas are still incomplete[4].

Poultry poses as the main reservoir for *Campylobacter* spp. harbor them without clinical manifestations and considered an important source of human illness [5]. Hence, the principle risk factors linked with Campylobacteriosis infection in human is the transmission of *Campylobacter* to humans either by handling or consumption of contaminated chicken meat and its products[6-8]. *Campylobacter* usually colonization the intestinal tract of chicken one week after hatching[9], however, the contamination of chicken meat contributed to cross contamination by intestinal contents at the slaughterhouse[10]. The ability of *Campylobacter* to colonize the chicken varies significantly not only among *Campylobacter* strains but also depending on the original source of the infecting isolate[11].

A wide range of genotypic methods with a high discriminatory power have been developed for *Campylobacter* typing[12]. Pulsed-field gel electrophoresis (PFGE) and MLST are the most widely used genotyping methods by laboratories worldwide for better understanding the epidemiology of *Campylobacter*. The MLST is considered one of the most important techniques elucidating the genetic diversity of *Campylobacter* isolated from animals and providing important information on transmission routes from different sources[13].

The MLST technique depends on sequencing of seven housekeeping genes to study the changes in ST distribution worldwide[14]. Subsequently, sequence data submitted to MLST database for assignment of the sequence type (ST) and clonal complex (CC) and to assign patient isolates to possible sources. In various studies conducting MLST, chicken was found to be the most frequent source of campylobacteriosis worldwide, representing from 38-77%, followed by cattle with an attribution rates varying from 16-54% [5].

Currently, detailed epidemiological information that determines the prospective sources of human campylobacteriosis in Egypt is not available and it is unclear whether same strains of higher risk are responsible for disease in Egypt. Thus, this study was designed to assess the frequency of thermophilic *Campylobacter* in broiler meat and to discover sequence types (ST) distribution of *C. jejuni* in broiler meat.

MATERIAL AND METHODS

Samples collection: A total of 190 broiler meat samples (chicken meat with the skin from breast, neck and thigh) were collected in the period between January and March, 2017 from six retail shops located at Mansoura city, Egypt. Each individual sample was separately packaged and transferred to the laboratory in an ice box within 1 h for bacteriological examination.

Bacteriological examination: Isolation of *Campylobacter* was conducted according[15] to ISO 10272-1. In brief, about 25 g from each chicken meat sample was pre-enrichmented in 225 mL Bolton Broth (Oxoid) supplied with SR0183 (Oxoid) for selective growth of *Campylobacter*. The inoculated broth was incubated firstly at 37°C for 4-6 h under microaerophilic condition by using CampyGen (Oxoid) and then at 42°C for 48 h. A loopful of the previously inoculated broth was plated on the surface of Modified Charcoal Cefoperazone Deoxycholate Agar (CM0739; Oxoid) supplied with SR0155 (Oxoid) and incubated for 48 h at 42°C under microaerophilic condition. Purification of *Campylobacter* colonies were performed onto Columbia Blood Agar (ASC; Biolife, Milan, Italy) containing 5% defibrinated horse blood and incubated at traces of oxygen (CampyGen, Oxoid) for 48 h at 41.5±1°C. Presumptive colonies were picked and stained with gram stain and tested with catalase, oxidase and motility tests.

DNA isolation: *Campylobacter* colonies were suspended in PrepMan Ultra (Applied Biosystems, Foster City, USA), the suspension heated at 95°C for 10 min for cell lysis followed by centrifugation. The supernatant containing bacterial DNA was transferred to a new tube and kept at - 20°C to be used as a DNA template for m-PCR[16].

Molecular characterization of *C. jejuni* isolates: Multiplex PCR assay (m-PCR) were developed[16] for detection of both *C. jejuni* and *C. coli* simultaneously. Three sets of primers were designed for detection of the following loci: 16SrRNA gene for co-identification of *C. coli* and *C. jejuni*, MapA gene specific for *C. jejuni* and CeuE gene encoding lipoprotein of enterochelin transport system characteristic for *C. coli* (Table 1). The PCR reaction and cyclic condition were performed following the protocol illustrated by Denis *et al.*[17].

Table 1: Oligonucleotide primers used in Multiplex PCR

Primers	Primer sequences	Amplicon	References
16S rRNA	MD16S1 upper primer 5'ATC TAA TGG CTT AAC CAT TAA AC 3'	857 bp	Linton *et al.*[18]
	MD16S2 lower primer	*Campylobacter* genus	
	5'GGA CGG TAA CTA GTT TAG TAT T 3'		
MapA	MDmapA1 upper primer 5'CTA TTT TAT TTT TGA GTG CTT GTG 3'	589 bp for *jejuni* species	Stucki *et al.*[19]
	MDmapA2 lower primer 5'GCT TTA TTT GCC ATT TGT TTT ATT A 3'		
CeuE	COL3 upper primer 5'	462 bp for *coli* species	Gonzalez *et al.*[20]
	AAT TGA AAA TTG CTC CAA CTA TG 3'		
	MDCOL2 lower primer 5'		
	TGA TTT TAT TAT TTG TAG CAG CG 3'		

Table 2: Oligonucleotide primers sequences used in MLST for *C. jejuni*

Primers	Sequences	bp
*asp*A	Forward: A1 AAAGCTGCAGCTATGGC	Amplification
	Reverse: A2 AAGCGCAATATCAGCCACTC	
*gln*A	Forward: A1 TAGGAACTTGGCATCATATTACC	Amplification
	Reverse: A2 TTGGACGAGCTTCTACTGGC	
*glt*A	Forward: A1 GGGCTTGACTTCTACAGCTACTTG	Amplification
	Reverse: A2 CCAAATAAAGTTGTCTTGGACGG	
gly	Forward: gly-A1, 5'-GAG TTA GAG CGT CAA TGT GAA GG-3'	Amplification
	Reverse: gly-A2, 5'-AAA CCT CTG GCA GTA AGG GC-3'	
tkt	Forward: A1 GAGTTAGAGCGTCAATGTGAAGG	Amplification
	Reverse: A2 AAACCTCTGGCAGTAAGGGC	
pgm	Forward: A1 TTGGAACTGATGGAGTTCG	Amplification
	Reverse: A2 AAGAGCTTAATATCTCTGGCTTCTAG	
*unc*A	Forward: A3 AAAGCTGATGAGATCACTTC	Amplification
	Reverse: A2 GCTAAGCGGAGAATAAGGTGG	
*asp*A	Forward: S3 CCAACTGCAAGATGCTGTACC	Sequencing
	Reverse: S6 TTCATTTGCGGTAATACCATC	
*gln*A	Forward: S1 GCTCAATTCATGGATGGC	Sequencing
	Reverse: S4 GCATACCATTGCCATTATCTCCG	
*glt*A	Forward: S1 GTGGCTATCCTATAGAGTGGC	Sequencing
	Reverse: S6 CCAAAGCGCACCAATACCTG	
*gly*A	Forward: S3 AGCTAATCAAGGTGTTTATGCGG	Sequencing
	Reverse: S4 AGGTGATTATCCGTTCCATCGC	
pgm	Forward: S3 GCTTATAAGGTAGCACCTACTG	Sequencing
	Reverse: S2 TCCAGAATAGCGAAATAAGG	
tkt	Forward: S1 TGCACCTTTGGGCTTAGC	Sequencing
	Reverse: S4 ACTTCTTCACCCAAAGGTGCG	
*unc*A	Forward: S5 TGTTGCAATTGGTCAAAAGC	Sequencing
	Reverse: S4 TGCCTCATCTAAATCACTAGC	

Multilocus sequence typing: The PCR was performed for amplification of seven housekeeping genes according to Dingle *et al.*[21]. The PCR reaction was performed in 100 µL using the Applied Biosystems 96 well thermal cycler using cyclic conditions reported by Dingle *et al.*[21] (Table 2). The PCR products were purified using QIAquick purification kit (Qiagen, Germany) and sent for sequencing. Sequence data were analyzed by submitting the sequences to *Campylobacter* MLST website (http://pubmlst.org/campylobacter) for assigning sequence types and clonal complexes.

RESULTS

In this study, the prevalence rate of *C. coli* from the examined broiler meat samples was 13.15% (25/190) while, *C. jejuni* was 3.68% (7/190) with overall prevalence of 16.84%. Among the identified *Campylobacter* spp., *C. coli* frequency found to be higher than *C. jejuni*.

By MLST genotyping, among the seven *C. jejuni* isolates, 3 STs were identified (ST21, ST48 and ST464). These STs were assigned to 3 CCs (CC21, CC48 and CC464) already described before (Table 3). Amongst the identified sequence types, ST21 was identified in 4 isolates and predominating among *C. jejuni* isolates identified in this study, while, ST48 was identified in two isolates and one isolate was assigned to ST464.

DISCUSSION

The incidence of *Campylobacter* infection has increased worldwide in the past decade. Understanding the epidemiology of *Campylobacter* species aids in reducing

Table 3: Allelic profiles, sequence types (STs) and clonal complexes (CCs) for *C. jejuni* isolates

	Allelic profile								
Isolates	*asp*A	*gln*A	*glt*A	*gly*A	*pgm*	*tkt*	*Unc*A	STs	CCs
1	2	1	1	3	2	1	5	21	ST-21
2	2	4	1	2	7	1	5	48	ST-48
3	2	1	1	3	2	1	5	21	ST-21
4	2	1	1	3	2	1	5	21	ST-21
5	24	2	2	2	10	3	1	464	St-464
6	2	1	1	3	2	1	5	21	ST-21
7	2	4	1	2	7	1	5	48	ST-48

the disease burden[4]. Therefore, in this study we aimed to characterize *Campylobacter* for better understanding the epidemiology of *Campylobacter* in our area with focusing on broiler meat as a most important source for human cases. Thermophilic *Campylobacter* species including *C. coli* and *C. jejuni* are frequently isolated from poultry. Poultry meat represents a potential source for human campylobacteriosis via consumption of contaminated poultry meat[22]. In the current study, the prevalence of *C. jejuni* was slightly lower if compared with other Egyptians surveys[23,24,25]. Compering with other surveys worldwide, the prevalence rate of *Campylobacter* spp. from retail chicken meat was 29% in Pakistan[26]. However, *Campylobacter* spp. has been stated as the second most frequent bacteria from chicken meat in Europe with a prevalence rate of 33.3%[27]. Presence of *Campylobacter* spp. in chicken meat may be caused by the contamination of carcasses with feces and rinsing due to unhygienic slaughtering and processing operations. Differences in the prevalence rate of *Campylobacter* from different countries may arise from differences in the area, sampling, transportation and sensitivity of the detection methodologies.

Among the identified *Campylobacter* spp. from this study, *C. coli* frequency found to be higher than *C. jejuni*. In agreement with this observation, higher percentages of *C. coli* have been reported in many previous studies by Nobile *et al*.[28] and Mezher *et al*.[29]. Conversely, a higher *C. jejuni* isolates was reported in other studies by Hafez *et al*.[25] and Wassenaar and Newell[30]. In general, many surveys worldwide reported this variability in the percentage between *C. coli* and *C. jejuni* in broiler meat[6].

Campylobacter is characterized by heterogeneity and there are many typing techniques were developed for its typing[31]. In the current study MLST was used to determine the diversity of *C. jejuni* isolates. The MLST is widely used for genotyping of *Campylobacter* worldwide but it is still not commonly used in Egypt with lacking of information about the *Campylobacter* species Sts.

The MLST is an important technique having a high discriminatory power[32,33] used in population studies of *Campylobacter* spp. Furthermore, MLST sequence data can be easy to interpret via submitting of sequence data to the Campylobacter MLST website and it readily compared between laboratories[32]. On the other hand, it is expensive and complex technique, labor-intensive to perform in comparison with other typing techniques[34].

In this study, ST21, ST48 and ST464 were identified. Amongst the identified sequence types, ST21 was identified in four isolates and predominating among *C. jejuni* isolates identified in this study, while, ST48 was identified in two isolates and one isolate was assigned to ST464. The prevalence of *C. jejuni* in broiler meat differs widely between countries, in our study, CC-21 was the highest CC assigned followed by CC-48. These findings go in line with these reported in Belgium[35,36]. On ST-level, ST21 was the dominant sequence type among the tested *C. jejuni* isolates. Similarly, ST-21 was reports as the most frequent sequence type form broiler meat worldwide[37-39]. In contrast to these findings, CC21, ST21 was more common in dairy cattle than broiler sources in Lithuania[16]. In addition, ST464 has been identified in our study and it has been also identified from broilers in Spain and Belgium[40,41].

In many previous studies, ST-21, ST-48 and ST-464 were reported amongst the most common sequence types identified in both human and broiler carcasses isolates[39,41] which may poses a potential risk for human. Further studies should be performed to evaluate the risk factors of *Campylobacter* contamination in the Egyptian poultry production chain.

CONCLUSION

This study has determined the percentage of contamination of broilers meat with *Campylobacter* spp. in Egypt with predominance of *C. coli*. In addition, by performing MLST on *C. jejuni* isolates, the obtained results

showed that the identified sequence types were frequently reported in human illnesses which may pose a potential risk for the consumers. To the best of our knowledge, this study considered the first study in Egypt providing information on the distribution *C. jejuni* STs from poultry sources.

SIGNIFICANCE STATEMENT

This study highlights the importance of broiler meat as a significant source of human campylobacteriosis. In addition, genotyping of *Campylobacter* with MLST helps in better understanding the epidemiology and the transmission pathways of *Campylobacter* to decrease the disease burden.

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