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Zoonotic concern of *Campylobacter jejuni* in raw and ready-to-eat barbeque chickens along with Egyptian handlers and consumers via molecular and immunofluorescent characterization

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ABSTRACT

Raw and under cooked barbeque chickenswhich harboring pathogens of public health and zoonotic impact concerning consumers and handler employees. Campylobacter jejuni considers one of the most prevalent chicken-borne gastroenteric bacteria. This study spots light on this concept to indemnify zoonotic hazard of C. jejuni by molecular characterization and indirect fluorescent of Egyptian isolates from both chickens and human in contact. From various Egyptian governorates and clinics a total of 588 chicken visceral contents, eviscerated raw and barbeque chickens were collected from different restaurants. Plus, 96 samples from both symptomatic consumers with history of chickens poisoning and chicken handler. Samples were subjected to standard phenotypic identification of C.jejuni, and subsequently immunofluorescent technique (IFT) identification and genetic amplification by PCR using specific primers of hipO gene. The positive results were detected by IFT expressed by green fluorescence staining. PCR amplification of hipO gene. The overall positive ratio of C. jejuni in chicken was 59.2%, where the higher and the lower values were recorded with intestinal contents and barbeque tissues (72.1 and 32.1) respectively. The total positive ratio in contact personals was 51%.Wherever, the higher and the lower values were 75.9% and 40.3% corresponding to symptomatic consumers and handlers employees. Molecular characterization of chicken's isolates have shown identical fingerprints with human isolates at 323bp, signifying the high possibilities of zoonotic hazards of the collected samples. The present studycan be concluded that the high incidence of C. jejuni in raw and barbeque chickens incriminated in high infection rate within consumers and handlers' employees' .This provides background for the design of firm efficient control strategies.

Keywords: C. jejuni, chickens, symptomatic consumers, fluorescence, PCR**.**

INTRODUCTION

Campylobacter jejuni is gram-negative spiral microaerophilic bacteria, inducing one of the most notifiable gastroenteric foodborne zoonosis, affecting about 2.4 million people, with up to 15% of all human diarrheal cases every year [1, 2]. It has been confirmed in various animal reservoirs, but poultry and their products have been

recognized as the main source [3]. C. jejuni may survive on watery products for several days with a distinct seasonal pattern through the peak incidence in summer months [4]. The free-range chickens are presumed to be a common source of C. jejuni. However, analysis of 156 human and 682 avian strains demonstrated identical strains in chickens and humans in 70.7% of families, and 39% of human isolates from diarrhoeal and non-diarrhoeal cases were identical to a household chicken isolate [5]. The organism is isolated from infants and young adults more frequently than from persons in other age groups [CDC, 2014]. Most strains of C. jejuni have opportunistic characters, and produce a cholera-like enterotoxin that hinders the cells from dividing, simulate watery diarrhea, fever and abdominal cramping. The most important human complication of C. jejuni is Guillian Barre Syndrome [GBS] which is an acute demylenating disease of peripheral nervous system, paralysis of the limbs which lasts for several weeks, also, include toxic megacolon, dehydration and sepsis specially in children [< 1 year of age] and immune-compromised patients [6]. USDA researchers have noted that most retail chicken is contaminated with C. jejuni with an isolation rate of 98% for trade chicken meat. C. jejuni counts often exceed 10^3 per 100 g. Skin and giblets have particularly high levels of contamination. So, In 2013, the UK's Food Standards Agency warned that two-thirds of all raw chicken bought from UK shops was contaminated with campylobacter, affecting an estimated half a million people annually and killing approximately 100 ,because of the "improper handling of foods by consumers and food service employees [7]. Culture-based methods are time consuming and expensive, requiring filtration, selective enrichment, isolation and biochemical confirmation [~9 days to report]. The application of molecular tools, such as PCR, may help to circumvent some of the limitations of current methods, where the hipO gene is specific for C. jejuni strains [8]. PCR targeting hipO gene was used previously for identification C. jejuni in raw, under cooked chickens, and human in contact [9]. This study was focused on the recognize of C. jejuni as a serious zoonotic pathogen, via describing the genetic and fluorescent characteristics of collected isolates from Egyptian raw and under cooked ready to eat chickens along with the suffering personnel and employees, reflect on improvement in food safety measures.

MATERIALS AND METHODS

2.1. Setting

The work was done in Zoonotic Diseases Department and unit of Biotechnology-Animal Reproduction Department, National Research Center, Egypt, from January 2013 up to July 2014.

2.2. Samples collection

2.2.1. Chicken Samples

588 chicken samples [219 chicken intestinal content, 161 chickens liver, 127 chilled chicken and 81 Barbeque chickens [core portions], were collected from different markets and restaurants of Alexandria, Cairo, Giza and Bin-suef governorates of Egypt.

2.2.2. Human Samples

Stool samples were collected from 96 persons; 67 were in contact with chickens [handlers employees], from different markets and restaurants, and 29 were symptomatic consumers with history of food poisoning of poultry origin collected from the governmental hospitals or health unites from the same governorates mentioned above [Table 1]. All samples were aseptically placed in separate sterile plastic bags and were immediately transported to the laboratory in a cooler with ice packs and processed immediately upon arrival for isolationofCampylobacter.

2.3. Isolation, purification and Identification

About 10 g of each sample were homogenized in sterile thioglucolate broth. Broth samples were incubated at 42 °C for 48 hrs. Under microaerobic condition [5% O_2, 10% CO_2 and 85% N_2]. A loopful of enrichment broth were plated on semisolid thioglucolate broth [Oxoid] and incubated in microaerophilic atmosphere at 25° C, 37°C and 42 °C for 48 -72 hrs. Microscopic examination of suspected colonies of Campylobacter were stained with Gram's stain and identified under phase contrast microscope using [1000 x] magnification power as cited by [10, 11] for detection of characteristic comma , S - shape and spiral motility characters of the isolated campylobacter organisms and deep stab growth, typical growth ring test. According to Hald et al. [12] suspected colonies plated onto blood agar plates. Campylobacter isolates were subcultured and identified by biochemical tests including catalase production test, nitrate reduction test, hydrogen sulphide production using lead acetate paper, glycine tolerance test, sodium chloride [NaCl] 3.5% tolerance test, Hippurate hydrolysis test and sensitivity to nalidixic acid and cephalothin. Identified colonies were stored at -70 °C in nutrient broths with 15% glycerol until subjected to molecular identification [13].

2.4. Indirect Fluorescent Antibody Techniques

Immunofluorescent identification of Campylobacter jejuni: The identified Campylobacter jejuni were prepared after Harlow and Lane [1988] and about [20 µl] is applied in duplicate to microscopic slides and prepared for immunofluorescence technique according to Mellick et al. [14] glass slides fixed in ethanol at 18-25 ^{o}C for 30 minutes were air dried and add antibody for C. jejuni were prepared by intramuscular injection in rabbit with 2 ml of 10^{11} organisms/ml of a C. jejuni [15, 16]. Sample slide carried out in a humid chamber at 37°C for 30 minutes in incubator. Subsequently, the slides are washed two times for 10 minutes in PBS and one time for 10 min. in distal water. Then added Antirabbit fluorescein isothiocyanate isomer [FITC]-conjugated antiserum. Staining is carried out in a humid chamber at 37°C for 30 minutes in incubator. Then, the slides were washed three times for 10 minutes in PBS. The slides are mounted in buffered glycerol [90% glycerol: 10% PBS]. The cover-slips are sealed to prevent drying, and the slides are examined under ultraviolet light in an epifluorescent microscope. Samples that show green fluorescent typical morphology of C. jejuni are considered positive.

2.5. Molecular characterization of Campylobacter jejuni

2.5.1. Isolation of DNA

DNA extracts were prepared for each isolate by using commercial DNA Extraction Kit [ViVantis Co., Malaysia]. The DNA pellet was dissolved in 50µl of elution buffer. Extraction of Genomic DNA from C.jejuni as mentioned above for use as a positive control. The crude DNA preparation was stored at 4°C until used.

2.5.2. DNA amplification reaction

PCR reaction contained 5ul template DNA and 1µl hipO primers [0.3 µM] [17], CJF [ACTTCTTTATTGCTTGCTGC] and CJR [GCCACAACAAGTAAAGAAGC] was Performed in a total reaction volume of 50 UL containing 25 µl Taq PCR master mix [ViVantis Co., Malaysia]. Thermo cycler conditions were 95 ^{o}C for 6 min, followed by 30 cycles of 94 ^{o}C for 30 s, 59 ^{o}C for 30 s and 72 ^{o}C for 30 s and finally 72 ^{o}C for 7min. positive controls was incorporated with each set of test samples and subjected to PCR assays. The PCR amplified products were loaded onto gels of 1.5% agarose gel and stained with ethidium bromide electrophoresis and visualized under UV transilluminator against 100 bp plus DNA marker [Finzyme]. The positive results were indicative at 323bp.

RESULTS

Samples were collected from Alexandria, Cairo, Giza and Beni-Suef governorates of Egypt for isolation of Campylobacter jejuni from chicken's intestinal contents, liver, chilled and barbeque chickens in addition to symptomatic consumers and handlers employees [Tables 1-3]. The samples were considered positive with C. jejuni on the base of the two identification tests [PCR & IFT]. In chicken samples, clear variation reference to different governorates [Table 2]. The total positive percentage of C. jejuni in chicken samples was 59.2%, with varies values of 72.1, 62.2, 52.8 & 32.1] for intestinal contents, liver, chilled chickens and barbeque chickens respectively [Table 2]. Observable dissimilarity was recorded within symptomatic consumers and handlers employees' reference to different governorates [Table 3]. The total positive percentage in contact personals was 51%.wherever, the higher values [75.9% & 40.3%] corresponding to symptomatic consumers and handlers employees [Table 3]. The estimation of C. jejuni carried out by deep stab growth, typical growth ring test on semisolid thioglucolate broth, and identification of characteristic comma, S - shape, and spiral motility characters.PCR amplification of the 323 bp products of DNA extracted from C. jejuni [Figure 1]. Also growth colonies observed onto blood agar plates .in addition to green fluorescence staining by IFT shown in [Figure 2].

Table 1. The collected samples from different Egyptian Governorates

Governorates	Chicken samples					Human samples		
	Total	Intestine	Liver	Chilled tissues	Barbeque tissues	Total	Workers in contact	Symptomatic consumers
Alexandria	174	60	45	42	27	26	17	9
Cairo	207	75	56	47	29	33	25	8
Giza	130	48	36	27	19	21	14	7
Bin-suef	77	36	24	11	6	16	11	5
Total	588	219	161	127	81	96	67	29

Table 2. Results of positive samples and ratio of C. jejuni isolated from chicken samples

Governorates	Chicken samples									
	Total		Intestinal contents		Liver		Chilled tissues		Barbeque tissues	
	n	+ve [%]	n	+ve [%]	n	+ve [%]	n	+ve [%]	n	+ve [%]
Alexandria	174	102 [58.6]	60	47 [78.3]	45	23 [51.1]	42	26 [61.9]	27	6 [22.2]
Cairo	207	126 [60.8]	75	54 [72]	56	32 [57.1]	47	29 [61.7]	29	11 [37.9]
Giza	130	73 [56.1]	48	33 [68.8]	36	18 [50]	27	17 [62.9]	19	5 [26.3]
Beni-Suef	77	47 [61]	36	24 [66.6]	24	12 [50]	11	7 [63.6]	6	4 [66.6]
Total	588	348 [59.2]	219	158 [72.1]	161	85 [52.8]	127	79 [62.2]	81	26 [32.1]

Table 3. Results of positive samples and ratio of C. jejuni isolated from chicken handlers and symptomatic consumers

Governorates	Human samples						
	Total		Chicken handlers		Symptomatic consumers with history of chickens poisoning		
	n	+ve [%]	n	+ve [%]	n	+ve [%]	
Alexandria	26	14 [53.8]	17	8 [47]	9	6 [66.7]	Sporadic cases
Cairo	33	19 [57.6]	25	11 [44]	8	8 [100]	Outbreak
Giza	21	9 [42.9]	14	5 [35.7]	7	4 [57]	Sporadic cases
Beni-Suef	16	7 [43.8]	11	3 [27.3]	5	4 [80]	Sporadic cases
Total	96	49 [51]	67	27 [40.3]	29	22 [75.9]	Sporadic cases

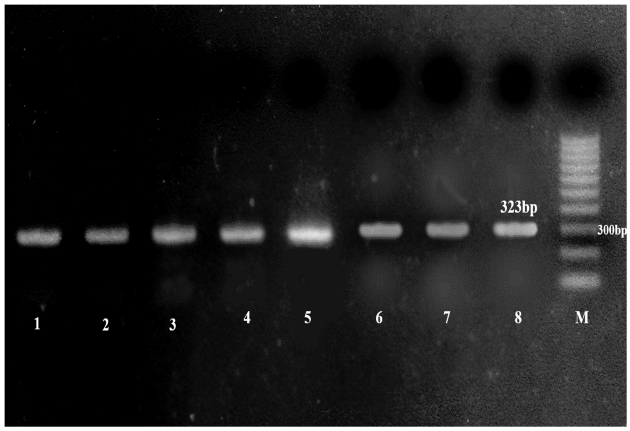

Figure 1. PCR amplification of the 323 bp products of DNA extracted from C. jejuni. Lane M: a 100 bp molecular size marker. Lanes 1-4, are C. jejuni isolates from chicken samples, and 5-7, human samples respectively. Lane 8: positive control

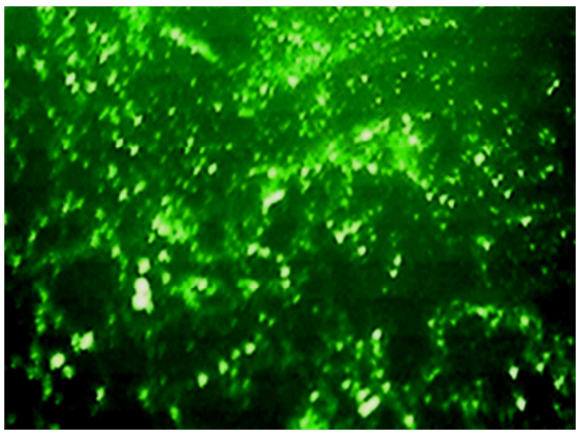

Figure 2. Positive C. jejuni isolated by IFT

DISCUSSION

The zoonotic Campylobacter jejuni is one of the most poultry harboring pathogens, with high public health hazard usually associated with chickens, sequence to the superior levels of human consumption [3]. The current study confirm the zoonotic hazard within the symptomatic consumers [75.9%] which were recorded higher percentages than the handler employees 40.3% [Table 3], denote that human infections occur mainly through consuming contaminated poultry [11]. But, shedder poultry duringslaughter or carcass dressing possible infect handler and in contact especially whose having skin abrasions [16]. furthermore, poor hygienic measures maximizing the common routes of transmission from polluted chickens via fecal-oral, person-to-person, ingestion of polluted food and water [3]. The high prevalence of C. jejuni in contact personnel's [51%] may be attributed to the high incidence of infected chickens. Other studies were verifying our results, where a survey in Cairo, Egypt determined the prevalence, seasonality, and household risk factors for Campylobacter-associated diarrhea in children; Campylobacter spp. were more prevalent associated with keeping fowl in the home [18]. The higher incidence of C. jejuni in consumers and handlers possible to either initial bacterial contamination over the permissible limit or improper application of naturals and spices all through chilling store phase [19]. Where only ten to five hundred bacteria are enough to infect humans [3]. Recently, a Campylobacter infection was detected in Egyptian personals [12.3%] that were exposed to infected backyard poultry [20].

In the present study the overall positive chicken samples which harboring C. jejuni were 59.2% [Table 2]. Our result is higher than C. jejuni isolated from that of Khalifa, et al. [36%], and El-Tras, et al. [23.5%] in chicken and poultry [9, 20]. The differences in the prevalence can be attributed to several factors, including isolation methods, sample types and size in addition to seasonal and regional variations [21]. Recently, high incidence of C. jejuni in infected raw poultry in Egypt [11].The current study set varies values with different types of chicken samples. however, the intestinal contents showed the higher values [72.1%], and barbeque chickens recorded the lower ones [32.1%], while chilled chickens and liver samples recorded 62.2% and 52.8% respectively [Table 2], this may explain the increase in the incidence of C. jejuni in poultry meat, as it is frequently polluted via either initial contamination from farm origin or pollution during processing via preparing utensils or food handlers.Also, dissimilar incidence was recorded for C. jejuni concerning under-cooked barbequed chickens which may be due to the varieties of treatment methods which differ in temperature degrees and pH or spices[17]. However, socio-economic difference via four governorates was none negligible factor which represented in cities difference and in hygienic measures applied during preparation or cooking. Poor sanitation in poultry farms could explain this high level of chickens harboring Campylobacter. Indeed, most farms do not have security fence to prevent penetration of other animals including rats, which are good carriers of Campylobacter. Furthermore, poor hygiene measures during process of slaughter possibly contaminate poultry carcasses, cleaning and disinfection of water-line between flocks may help to reduce the risk of chicken Campylobacter colonization [22].Different governorates had signifying clear dissimilar values of chicken samples harboring C. jejuni [Table 2]. Also, show obvious dissimilar values within both symptomatic consumers and handlers employees within the four governorates [Table 3].This variation among four the governorates may be due to geographical, warm or cold weather in addition to population factors [23]. Higher temperature and humidity enhance Campylobacter growth [24, 25]. The reason is still debated but may indicate a possible relationship between temperature and Campylobacter survival and transmission as stated by Patrick et al. [26]. Also, insects frequently engaged in summer season [higher temperature] may be an important source of Campylobacter infection via mechanical transmission, where flies, cockroaches and other insects passed through the ventilation system into the chickens' house and the invasion of insects was correlated with the outdoor temperature [12].

In our study, PCR amplification of C. jejuni isolated from chicken showed identical fingerprints with human isolates, these diagnostic DNA bands of based on hippuricase gene amplified at 323bp [Figure 1] , in accordance with Wang et al. [17]. A finding substantiates our previous uses of hipO gene in molecular study of isolats from chicken and human to determine their zoonotic importance [27] and molecular characterizations of C. jejuni [9].

CONCLUSION

The present study spot light on the public health and the need for enhanced efforts at the surveillance for better control of zoonotic C. jejuni. Based on the results, it can be concluded that the high incidence of C. jejuni in raw and barbeque chickens incriminated in high infection rate within consumers and handlers' employees' .This provides background for the design of firm efficient control strategies.

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