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Prevalence of *Campylobacter jejuni* in fresh meat products

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ABSTRACT

Campylobacter jejuni (C. jejuni) is one of great significance to public health as it is the main source of food borne *Campylobacter* enteritis in human. Raw and undercooked contaminated meat products are known as important sources of human campylobacteriosis. Contamination of meat can occur in different steps during production as preparation, processing, distribution, marketing and handling at transportation. The main object of study was to isolated and identified of *C. jejuni* in from fresh meat by conventional methods and confirmed by polymerase chain reaction (PCR). Sixty one samples were collected from fresh meat including minced meat (15), liver (14), meat (25) and sausage Baladi (7). The prevalence rate of *C. jejuni* in fresh meat by PCR was 32.7% compared with conventional methods (19.67%). Polymerase chain reaction targeting hip *O* gene specific for *C. jejuni* was used for phenotypically identified *C. jejuni* isolates. This study concluded that PCR was more specific and rapid than the conventional methods for identification of *C. jejuni* in fresh meat. Raw retail meats need control programs and consumer food safety education efforts by application of hygienic measuring.

1. INTRODUCTION

C. jejuni is a gram-negative bacterium. It is considered one of the most important from both a microbiological and public health perspective (Ryan and Ray, 2004), can cause infection and gastroenteritis (Brownsell *et al.,* 1989). *Campylobacter* species are widespread in most warm-blooded animals. They are prevalent in food animals such as poultry, cattle, pigs, sheep, ostriches, and shellfish as well as pets, including cats and dogs (World Health Organization, 2011). Consumption of contaminated or undercooked meat (especially poultry) is the major route of transmission in humans, (Center for Food Security and Public Health, 2013). Contamination of meat can occur in different steps along the food production chain including production, processing, distribution, marketing and handling or preparation (Zhao *et al*., 2001). Thermophilic *Campylobacters; C. jejuni, C. coli, C. upsaliensis* and *C. lari* implicated as food borne infections (Iovine*et al*., 2008). *C. jejuni*is the most of the food borne enteritis in human, followed by *C. coli* and lesser extent *C. lari* (Skirrow and Blaser, 2000). *C. jejuni* has been frequently reported as a cause of human campylobacteriosis (80-90%) compared to *C. coli* (5-10%) (EFSA, 2008)

Detection of thermophilic *Campylobacter* species based upon performing catalase test, susceptibility to nalidixic acid and cephaloxcin and rapid hippurate hydrolysis test. Most of *Campylobacter* isolates were resistant to nalidixic acid, so that it is used in the distinction between *C. jejuni, C. lari* and *C. coli* (Megraud, 1987). Hippurate hydrolysis is the only phenotypic exam differentiating *C. jejuni* from other species of *Campylobacters,* especially the thermophilic species. *C. jejuni* is capable of hydrolyzing sodium hippurate to benzoic acid and glycine (ISO, 2006).The main object of this study was to isolate and identify of *C. jejuni* from fresh meat by conventional methods and confirmed by polymerase chain reaction (PCR).

2. MATERIAL AND METHODS

Our study was performed during the period between August 2017 and January 2019 in Reproductive Diseases Department, Animal Reproduction Research Institute, ARC, El Haram, Giza

2.1. Samples

A total of 61 samples gathered from fresh meat products including minced meat (15), liver (14), meat (25) and sausage Baladi (7) from many supermarkets and slaughterhouses in Cairo Province

2.2. Culturing of prepared samples

All samples were homogenized and inoculated in thioglycolate broth then incubated at 42 ° C for 48 hrs under microaerobic condition (5% O_2, 10% CO_2 and 85% N_2) (Gebhart*et al*. 1985).

A loopful from each sample were cultured directly on thioglycolate broth medium and then sub-cultured in modified *Campylobacter* blood free selective medium. The plates were incubated in microaerophilic condition (CO_2 (10%), O_2 (5%) and nitrogen (85%) at 37°C for 48 hours (Smibert, 1974).

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2.3. Biochemical identification of Campylobacter colonies

Identification of suspected colonies was done depending on characteristics colony morphology, Gram stain Koneman *et al*.,(1995), motility test (Smibert, 1974)and biochemical tests (Catalase production test, Nitrate reduction test, Oxidase test, Urease test, H_2S production test with lead acetate paper, Temperature tolerance test, Glycine tolerance test, NaCl tolerance test, Hippurate hydrolysis test, sensitivity to cephaloxcin and nalidixic acid) Al-Gohary, (1998).

2.4. Molecular identification by PCR

C*ampylobacter* DNA was extracted by using (Thermo Scientific Gene Jet Genomic DNA Purification Kit#K0721, #K0722).Species-specific primer targeting *hip O* gene specific for *C. jejuni* (Wang *et al*., 2002) were 5` ACTTCTTTATTGCTTGCTGC3`(forward) and 5`GCCA-CAACAAGTAAAGAAGC3`(reverse). PCR amplification was done by using thermal cycler (Biometra) (Wang *et al*., 2002). 1.5% agarose gel (Biometra) was used for electrophoresis of the amplified PCR products. The gel was photographed by Alpha Innotech system (El-Adawy*et al*, 2012).

3. RESULTS

Bacteriological examination of collected samples revealed isolation of *C. jejuni* from 12/61 (19.67%) fresh meat product samples. They were isolated from minced meat (5/15) (33.3%), meat muscles (5/25) (20%), sausage baladi (1/7)(14.3%) and liver (1/14)(7.1%). The samples confirmed by molecular PCR targeting hip *O* gene specific for *C. jejuni*. The occurrence of *C. jejuni* in fresh meat by PCR was 20/61 (32.7%) compared with conventional methods 12/61 (19.67%).The highest percentage for isolation of *C. jejuni* was in minced meat 33.3% by CM and 46.6% by PCR, the isolation rate of *Campylobacter jejuni* from sausage was 14.3% by CM and 28.6% by PCR. The results of *C. jejuni* in meat muscles were 20% by CM and 28% by PCR but, the incidence of *C. jejuni* in liver was 7.1% by C.M. and 28.6% by PCR (Table, 1 and Fig. 1).

Table (1): Incidence of *C. jejuni* in fresh meat product samples by CM and PCR

Type of Samples	No. of Samples	Fresh meat samples			
		Positive by CM		Positive by PCR	
		No	%	No	%
Minced meat	15	5	33.3%	7	46.6%
Liver	14	1	7.1%	4	28.6%
Meat	25	5	20%	7	28%
Sausage Baladi	7	1	14.3%	2	28.6%
Total	61	12	19.67%	20	32.7%

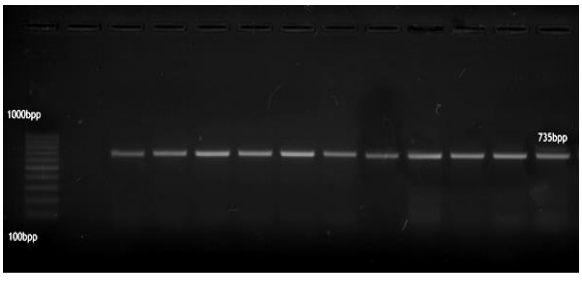

Fig (1): Electrophoresis profile of *C. jejuni* producing amplicons of average size 735 bp for hip gene, lane 3 positive control *Campylobacter jejuni* (ATCC 33291)

4. DISCUSSION

Campylobacters are one of the most important food bacteria leading to gastroenteritis in humans in developed and developing countries (Rahimi and Ameri, 2011).). *Campylobacter jejuni* causes more than 90% of the reported *Campylobacter* infections (NARMS, 2010). *C. jejuni* is distinct by rapid Sodium hippurate hydrolysis test which is the only phenotypic test differentiating *C. jejuni* from other species of *Campylobacters*, especially thermophilic species (ISO, 2006). Moreover, PCR targeting *hip* O (benzoglycineamidohydrolase) has been used to confirm the detection and discrimination of *C. jejuni*. The gene *hip* O is specific for the hippurate activity and differentiates *C. jejuni* from other *Campylobacter* species (Englen et al., 2003).

In this current study, the overall occurrence of Campylobacter jejuniwas19.67% (12/61) fresh raw meat samples by conventional methods while by PCR, its prevalence was 32.7% (20/61). The largest incidence rate of *C. jejuni* was identified in minced meat (5/15)33.3%, followed by meat (5 /25) 20%, Baladi sausage (1/7) 14.3% and liver (1/14) 7.1%.(Table, 1).

Nearly similar prevalence rate of *C. jejuni* from beef meat obtained at retail outlets was 22% of examined samples (Datta et al., 2003). Also, the prevalence of *Campylobacter* SPP. isolate was 16.66% in beef meat, 26.66% in beef liver and 22% in pediatric diarrhea. *C. jejuni* were 60% higher than *C. coli* (Parkhill et al.2000).Elgabry et al., (2016) recorded 16% (117/733) from fast meat meals (shawarma, kofta, hamburger, sausage, offal, and liver) in five Egyptian governorates. The distribution of *C. jejuni* from fast meat meals was 25.3%(19/75) in Assuit, 16.8%(28/167) in Fayuom, 15.3%(26/170) in Qaluobia,14.7%(30/204) in Cairo, and 12%(14/117) in Bin-suef (Elgabry et al.,2016).

Lower isolation rate of *C. jejuni* from beef meat was obtained in Great Britain; 1.6% from meat samples (Turnbull PC and Rose P. 1982),U.S. Washington; 4.7% (6/129 bovine meat) (Stern et al., 1984), Poland;0%(0/114 beef) (Kwiatek et al., 1990), Assuit; 12% (3/25 slaughtered buffalo) (Refaie and Galal, 1991), the Greater Washington, D.C., area, including suburban Maryland; 0.5% (1/182 beef samples) (Zhao et al., 2001) ,Istanbul, Turkey; 3% (6/198 beef samples from carcasses) (Bostan et al., 2009), Northern Poland; 10.1%(35/347beef meat) (Andrzejewska et al., 2019),Italy; 0;.24% (3/1203 bovine meat samples (689 hamburgers and 514 knife-cut meat preparations) (Giannatale *et al.*,2019) Africa;4% (21/521cattle meat) 4.47% (37/827 cattle carcass) (Thomas KM *et al.*,2020), Mekelle, Ethiopia; 9%(19/210 cattle meat) (Hagos *et al.*, 2021), and in Belgium beef carcasses and cutting meat(3.3% and 5.0%, respectively) (Ghafir et al., 2007). Several studies recorded absence of *Campylobacter* isolation in examined samples. As, examination of133 retail beef samples, consisting of ground beef and whole muscle steaks and roasts in a rural Midwest city (Fargo) (Kegode et al., 2008); retail ground beef samples (n ~ 100) in Alberta, Canada (Bohaychuk, *et al.*, 2006); a survey on beef carcasses conducted in 10 abattoirs in Northern Ireland (Madden et al., 2001) and from veal carcasses in Belgium (Ghafir et al., 2007).

However, higher findings of *C. jejuni* from beef meat were reported in Ghana 29.1% (32/110 cattle carcasses) (Karikari et al., 2017), Ottawa, Ontario 50% (50/100). The distribution form of *C. jejuni* -positive animals, in decreasing order, was steers (55%) (42/76), bulls (40%) (4/10), heifers (40%) (2/5), and cows (22%) (2/9) (Garcia et al., 1985), Belgium100% of isolates were *C. jejuni* (5/5beef meat) between 2000 and 2003, (Ghafir et al., 2007).

The occurrence rate of *C. jejuni* in beef livers in our study was 7.1% (1/14 beef liver) by conventional methods and

28.6% (4/14 beef liver) by PCR. That was higher than reported in Brazil; 1.51% (2/132 chilled beef liver) (Takeuchi et al., 2022), and Turkey; 0%(0/325 Liver samples from apparently healthy cattle by PCR) (Açik and Çetinkaya, 2005).From the other hand, our isolation rate was lower than the previous studies conducted in *Washington, D.C.;*15%(6/40 fresh beef liver) (Stern *et al.,* 1984), Ottawa, Ontario; 12%(12/100) (Garcia *et al.,* 1985), Assuit Governorate;8%(2/25 fresh liver samples) (Refaie and Galal., 1991), Toukh, Kaliobia governorate; 16.66% (5/30 beef liver) (Khalifa, Nashwa. (2013)), Egypt; 18.2% (26/143 offal and liver) (Elgabry*et al.,*2016), 30% (3/10) from offal and liver was in Assuit, 22.9%(8/35) in Qaluobia, 21.2% (7/33) in Fayuom, 12.8%(5/39) in Cairo and 11.5%(3/26) in Bin-suef (Elgabry et al.,2016), and Africa; 16.66%(5/30 cattle liver) (Thomas *et al.,*2020). Garcia et al., (1985) reported 12% (12/100) from the examined liver samples (Garcia et al.,1985). Noor mohamed and Fakhr, (2013) reported the overall prevalence of *C. jejuni* in beef livers was 26% (13/50 beef livers) and 2/50 (4%) of the samples was contaminated with both *C. jejuni and C. coli.* Also, Ghafir et al., (2007) suggested that the highest *C. jejuni* recovery from livers probably because the liver surface stays moist suitable for foodborne pathogen. Fecal contamination of *C. jejuni* by slaughtered cows is a possible source of contaminated beef liver.

Although the overall prevalence of *C. jejuni* in minced meat in the present investigation was 33.3% (5/15) by conventional methods and 46.6% (7/15) by PCR, no positive result was found from minced meat from beef and veal in Belgium. Pork and beef minced meat were contaminated with *Campylobacter* at a very low level: on average, 2.5% and 0.6% in 25 g samples, respectively in Belgium (Ghafir *et al.,* 2007). Furthermore, *C. jejuni* was not isolated from chilled minced meat 0% (0/138) in Brazil (Takeuchi *et al.,* 2022). Moreover, only in one sample minced beef were both Salmonella and Campylobacter found together (Turnbull PC and Rose P. 1982).On the other hand; *C. jejuni* was isolated from all samples of minced meat. *It* is a common or regular finding in minced meat for sale in ordinary grocery stores (Svedhem et al., 1981). The isolation rate of *C.jejuni* in the current study in sausage baladi was 14.3% (1/7) by conventional methods and 28.6% (2/7) by PCR. That was in line with other study recorded by Elgabry et al.,(2016)in Egypt, who reported prevalence rate 15.2% (27/178 sausage) from five Egyptian governorates. The highest incidence rate was detected in Assuit 20.8%(5/24) followed by 15.4%(6/39) in Fayuom, 15.2%(7/46) in Cairo, 13.2%(5/38) in Qaluobia and 12.9%(4/31) in Bin-Suef.

The maximum identification percentages of *C. jejuni* by PCR was in minced meat (7 /15) 46.6% followed by sausage (3/9) 33.3% then, liver (4 /14) 28.6%, and meat muscles (7/25) 28% (Table,1 and Fig. 1).

The fluctuation in *Campylobacter* species isolation rate in various studies have be attributed to many reasons as, type of examined samples, location, climate factors, hygienic measures and isolation as well as identification techniques (Jorgensen et al., 2011and Chatur et al., 2014). In addition to technical differences in protocols experiment, various other factors as size, breed of herd, season, animal ages, geography, or husbandry practices (Stanley and Jones, 2003 and Hakkinen and Hanninen, 2009).

5. CONCLUSION

Finally, it is concluded that the examined fresh meat was contaminated by *C. jejuni*. Consumption of undercooked or cooked contaminated meat products is considered a possible risk factor of human campylobacteriosis. Raw retail meats are potential vehicles for transmitting foodborne diseases, need control programs and consumer food safety education efforts by applied hygienic measuring, continued applying of HACCP systems, and increased consumer food safety at home.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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