Investigation of germ caused by Guillain-Barré syndrome in poultry meat products in North West of Iran

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Abstract

Campylobacter species are the most important pathogens that cause bacterial gastroenteritis being spread through food with animal origin. Given such fact, the current study aimed at investigate the prevalence of Campylobacter based on phenotypic and genotypic analysis of poultry meat and edible offal in Western Azerbaijan. To conduct the study, a total of 552 chicken samples including meat (samples), liver (138 samples), gizzard (138 samples) and hearts (138 samples) were randomly collected from poultry slaughterhouses at West Azerbaijan province from April 2014 to September 2014. Based on the culture tests, 208 samples (37/7%) were contaminated with *Campylobacter* species. The highest range of *Campylobacter* species out breaks was observed in poultry liver (49/2%), followed by gizzard (42/8%), heart (33/3 %) and meat (25/4%). Among the isolated *Campylobacter*, *C. jejuni* was the most prevalent (78/4%) and the rest were *C. coli* species (21/6%). All 208 species of *Campylobacter* isolated as *C. jejuni* and *C. coli* from species culturing were also approved by m.PCR. The results of the study pinpointed to the chicken edible offal importance as a potential source of *Campylobacterial* contaminations.

Introduction

Campylobacteriosis, serving as an important common disease, shoulders a major role in infectious gastroenteritis in humans and is considered as the world's first cause of gastroenteritis followed by dysentery and diarrhea and other complications such as meningitis, Guillain Barre syndrome and cholecystitis.[1,2]

Digestive disorders and diarrhea caused by the bacterial Campylobacter is among the common illnesses primarily in developing countries, and 5 to 15 percent of diarrhea in these countries are instigated by the bacteria, and the prevalence of the bacteria as a cause of diarrhea in our country has been reported to be from 2 to 10%,[3] and even the primary cause of death in developed countries, especially among children under 5 in the United States, was attributed to such bacteria in that two million cases of bacterial infection are reported each year.[2]

The bacteria which was first discovered in the nineteenth century by Theodor Escherichia a gram-negative, spiral-shaped or curved, growing and multiplying at Microaerophilic conditions at a temperature of 42°C, and below 25°C, it stops growing and multiplying but does not disappear.[2] The thermophilic Campylobacter species, such as Jejuni and Coli, have an important role in the development of Campylobacteriosis becoming more prominent in the food infection as the spread of Campylobacter contamination in food has become very important in recent years going even further than Salmonella.[2] Studies show that the bacterium originates mainly from food with animal resource. Moreover, raw milk, meat and poultry, untreated surface water and edible fungi are the sources of infections with Campylobacteriosis disease being common in spring and summer. Infections with Campylobacter have been reported in several countries in broiler chickens, so that the bacteria survived during the slaughtering process and were transmitted to uninfected poultry organs.[2] Due to its high sensitivity and detection speed, the PCR method has the potentiality to determine if there is even a bacterium per ml and reaches the response in a very short time. Many studies have examined the prevalence of the bacteria in meat and poultry as well as diarrheal samples.[4] The consumption of half-baked meat and the related products serves as the main source of human infection although other livestock meat and milk products are potential sources of the disease. There are reports on the prevalence of Campylobacter among live birds and animals and different kinds of food from all over the world revealing upon a wide range of results. Previous reports highlighted the infection in chickens from zero to 100% which was up to 60% for the cows. The prevalence of the infection in poultry given to the market have been reported to be 100% and lower incidences of other animals meat were also given.[5,6] Although there are many studies casting light on the prevalence of Campylobacter species among poultry in Iran,[7-10] and other countries including Korea,[11] Japan,[6] Canada,[12] Ireland,[13] Pakistan,[14] Belgium,[15] little data existed on the poultry meat by-products infection including liver, gizzard and heart in Iran, therefore, the study aimed at evaluating the contamination of these products.

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Materials and Methods

To conduct the study, a total of 552 samples including chicken meat (138 samples), liver (138 samples), gizzard (138 samples) and hearts (138 samples) were randomly collected from poultry slaughterhouses at West Azerbaijan province from April 2014 to September 2014and were transferred to the laboratory for analysis aiming at investigating the presence of Campylobacter species in cold temperature. To determine the phenotype of bacteria, 10 grams of the sample was added to a bag with one hundred mL broth Preston (Preston enrichment broth base, Himedia, Mumbai, India, m899) containing five percent defibrinated sheep blood for enrichment. The bags were incubated for 24 hours at 42°C in carbon dioxide presence. Then, the liquid in the bag was removed by a sterile loop and cultured on specific Campylobacter selective Agar (Himedia, Mumbai, India, m994) as a separate line to obtain single separated colonies. Then, it was incubated for 48 hours (Co2 10% v/v) at 42°C. After the initial plate analysis, the suspected colonies were stained with Gram's stain and upon the curved bacteria resolution, the re-culturing was also applied. Upon obtaining the colonies, the biochemical tests including catalase, Indoxyl acetate hydrolysis were used and in order to differentiate between the two species Jejuni and Coli hippurate hydrolysis

test was conducted, which was positive for Jejuni.[1]

To investigate the Campylobacter genotypic, DNA extraction and Multiplex PCR method were applied. Using the culturing method and DNA extraction kit (Cina Gen, Iran), the confirmed colonies DNA were extracted according to the Kit manufacturer's instructions. The PCR test in this study followed Denis *et al.* procedure.[16] For PCR reaction, the reaction final volume of 25 microlitres was considered including 20 ng DNA template, 2 mM MgCl2, 25 pmol of each primer, a single Taq polymerase enzyme and 200 mM mixed dNTP. The size of PCR products corresponding to each sample is given below. To confirm the presence of the multiplied sample, 20 microlitres of PCR was paced on electrophoresis with 1/5 percent agarose gel containing ethidium bromide in the presence of 100 bp DNA marker in constant voltage of 80 volts (Table 1).

Results

The current study aimed at evaluating Campylobacter phenotypic and genotypic outbreaks in chicken meat and its edible offal at West Azerbaijan province, Iran. To conduct the study, a total of 552 samples including chicken meat (138 samples), liver (138 samples), gizzard (138 samples) and hearts (138 samples) were randomly collected from poultry slaughterhouses at West Azerbaijan province from April 2014 to September 2014 and were transferred to the laboratory for analysis aiming at investigating the presence of Campylobacter species in cold temperature.

Based on the culture tests, 208 samples (37/7%) were infected with Campylobacter species. The highest range of Campylobacter species outbreaks was observed in poultry liver (49/2%), followed by gizzard (42/8%), heart (33/3%) and chicken meat (25/4%). Among the isolated Campylobacter, the Jejuni type was the most prevalent (78/4%) and the rest were of Coli type (21/6%). All 208 species of Campylobacter isolated as Jejuni and Coli types from culturing were also approved by multiplex polymerase chain reaction test (m.PCR). A statistically significant difference ($P<0.05$) was observed in Campylobacter species outbreak in meat samples taken in summer (40/9 %). The results are presented in Tables 2-4 and in Figure 1.

Discussion

The given result shows 208 samples (37/7%) were infected with Campylobacter species. Previous studies in Iran have reported the contamination level to the bacteria for different cities as Isfahan with 56/1 percent,[8] Shahrekord with 47 percent,[7] Tehran with 63/2% and 49/5%,[9] and Mashhad with 76%.[17]

The outbreak of Campylobacter species in chicken meat in other countries also suggests a contamination level of 30 to 90 percent. The contamination levels in different countries were as follows: Turkey with 92/8%,[18] Korea with 68/3%,[11] Canada with 62/4%,[12] Japan with 60%,[6] Ireland with 49/90%,[13] and Pakistan with 48%.[14] Despite having many studies reporting the prevalence of Campylobacter species contamination in poultry, there are few studies highlighting on the edible offal contamination to the bacteria.

A similar study conducted by Rahimiin 2006 and 2008 casted light on the prevalence of Campylobacter in the chicken liver marketed in Isfahan. Accordingly, the contamination of 205 samples under study was reported to be 49/3%, and the liver contamination for the, chicken, turkey and ostrich were 49/3%, 40% and 16/7%, respectively.[8] Also, Shakerian *et al.*[19] in a study on evaluating the Campylobacter contamination in poultry liver in Shahrekord indicated that

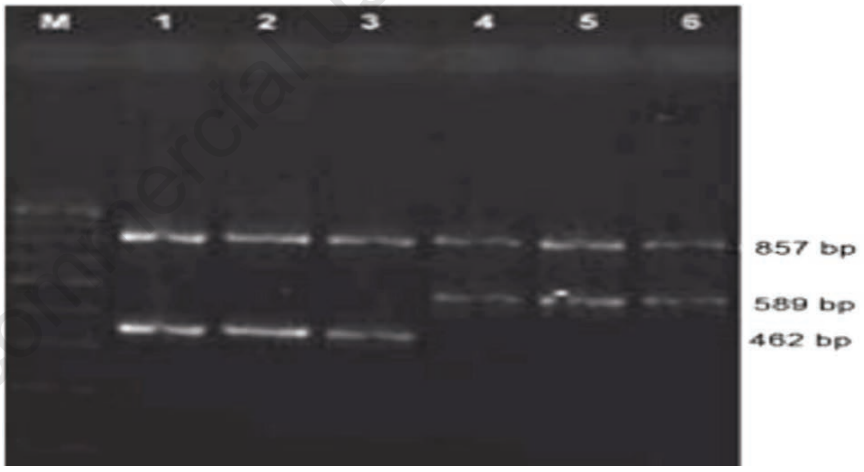

Figure 1. Multiplex PCR amplicons on 1.5% agarose gel. Lane M: 100 bp ladder; Lane: 1, 2, 3 - *C. coli*; Lane: 4, 5, 6 - *C. jejuni*.

Table 1. Sequences of primers used for detecting Campylobacter and the related *Jejuni* and *Coli* species.

Gene	Primer consequence	Type of product
16SrRNA	MD16S1 Upper Primer 3 ATC TAA TGG CTT AAC CAT TAA AC5 MD16S1 Lower Primer 3GGA CGG TAA CTA GTT TAG TAT T 5	857 bp for *Campylobacter* genus
mapA	MDmapA1 upper Primer 3CTA TTT TAT TTT TGA GTG CTT GTG 5 MDmapA2 Lower Primer 3GCT TTA TTT GCC ATT TGT TTT ATT A5	589 bp for *C. jejuni*
CeuE	COL3 Upper Primer 3AAT TGA AAA TTG CTC CAA CTA TG5 MDCOL2 Lower Primer 3TGA TTT TAT TAT TTG TAG CAG CG5	462 bp for *C. coli*

259 samples of 400 samples (64/8%) were infected with Jejuni Campylobacter. The report by Ghafir *et al*.[17] suggests that contamination of broiler chicken liver distributed in Belgium capital from 1997 to 1998 was about 68/7%. The chicken liver infection rate in 1997 and 1998 were 61/7% (74 from 120) and 74/6% (106 from 143).[15] Sallam *et al*.[20] (2007) reported the contamination of meat and edible offal for chicken from 40 to 77 for breast, thighs, wings, liver, gizzard and heart as 64/4%, 70%, 77/1%, 64%, 45% and 40%, respectively.

Suzuki and Yammamato (2009) reported the contamination of chicken meat, gizzard, liver and heart as 59%, 62/2%, 62/3% and 33/3, respectively.[6] In both studies similar to result of this study, the highest infection rates was in liver and the lowest was observed in heart. The reason could be due to liver greater contact area than the heart and its further manipulation. The differences between the results reported from different parts could be attributed to the poultry infection rates in different regions, the interval between studies, the difference in the killing and hygiene practices during different slaughtering phases, sampling seasons and sensitivity of testing methods.

The results showed that among the isolated Campylobacter, the Jejuni type was the most prevalent (78/4%) and the rest were of Coli type (21/6%). Other studies have also shown that Jejuni type is the most common species in food with animal origin.[6-8,14,17,21] For example, in a study by Hussain *et al*.[14] (2007) the prevalence of Campylobacter species (Jejuni and Coli) in food samples with animal origin was 70/6% and 29/4%, respectively. The same study reported the prevalence of Jejuni and Coli Campylobacter in chicken as, 72% and 28%, in sheep meat as 65% and 35% and in cow meat as 79% and 21%, respectively.

Another similar study in 2004 was conducted in Ireland by Whyte *et al*.[13] focusing on Jejuni and Coli Campylobacter in food with animal origin which revealed the fact that the contamination level for Jejuni and Coli type were 3804 and 16/6, respectively. The prevalence of bacteria in chicken as 6/84 and 6/16% have been reported.

Moreover, evaluating the Campylobacter contamination in poultry meat samples in different season showed there existed a significant difference in contamination level in summer (50/0>P) than in other seasons which was also confirmed by reports from other studies.[12] Such high prevalence could be attributed to high temperature creating favorable conditions for the bacteria growth of and transferring of infection by insects. The overall results of this study on chicken meat and its edible offal contamination to Campylobacter species showed that a relatively high number of samples especially the liver were infected with this pathogen. Therefore, in order to reduce contamination of chicken meat and its edible products to Campylobacter species and similar microorganisms, maintaining individual health, preserving sanitation in slaughterhouses, following HACCP principles in poultry chains, minimizing the carcasses contact with the edible offal, minimizing the chicken carcasses contact and maintaining the least manipulation and drinking water in slaughtering process seem to be the most important. Also, maintaining hygiene practices in splitting, packaging, and transportation stages and maintaining the cold condition in meat preserving chain until being delivered to consumer serve as very important measures in reducing meat contamination to such pathogens.

Table 2. The contamination of chicken meat and its related edible offal to Campylobacter and its species.

Samples	Number	Positive number (%) campylobacter infection	Positive number (%) campylobacter jejuni infection	Positive number (%) campylobacter coli infection
Meat	138	35 (4/25)	32 (4/91)	3 (6/8)
Liver	138	68 (2/49)	61 (7/89)	7 (3/10)
Gizzard	138	59 (8/42)	53 (8/89)	6 (2/10)
Hearts	138	46 (3/33)	42 (3/91)	4 (7/8)

Table 3. Monthly contamination of chicken meat and its related edible offal to Campylobacter and its species.

Samples	Number	Positive number (%) campylobacter infection	Positive number (%) campylobacter jejuni infection	Positive number (%) campylobacter coli infection
March	92	29 (6/31)	23 (3/79)	6 (7/20)
April	92	32 (8/34)	25 (1/78)	7 (9/21)
May	92	34 (37)	26 (5/76)	8 (5/23)
June	92	35 (38)	28 (80%)	7 (20)
July	92	37 (2/40)	29 (4/78)	8 (6/21)
August	92	41 (6/44)	32 (78)	9 (22)

Table 4. Seasonal contamination of chicken meat and its related edible offal to Campylobacter and its species.

Samples	Number	Positive number (%) campylobacter infection	Positive number (%) campylobacter jejuni infection	Positive number (%) campylobacter coli infection
Spring	276	95 (4/34)	74 (9/77)	21 (1/22)
Summer	276	113 (9/40	89 (8/78)	24 (2/21)
Total	552	208 (7/37)	163(4/78)	45 (6/21)

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