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Prevalence of *Campylobacter* spp. and their Common Serotypes in 330 Cases of Red-meat, Chicken-meat and Egg-shell in Zanjan City, Iran

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Background: *Campylobacter* spp. are the common pathogens that infect human beings via food. These bacteria are vibrio and have been implicated in abortion. Serotyping is the best way for typing with Penner scheme. *C. jejuni* and *C. coli* have 65 serotypes. *C. coli* is common in birds and dogs. Due to high rate of prevalence of *Campylobacter* in red-meat, chicken-meat and egg-shell, a suitable method to detect their prevalence, the most common species and serotyping group was necessary. This article describes the prevalence of *Campylobacter* infection, common serotyping group in 330 samples of red- meat, hen-meat and egg-shell.

Materials and Methods: With three methods: enrichment, selective Preston and Skirrow and filtration with membrane filters *Campylobacter s*were incubated. Bacterial species were identified with physiological and biochemical tests. Penner serotyping was defined with reference antiserum Ag-O and direct agglutination.

Results: Prevalence of *Campylobacter* infection was 21(23%) in red meat, 33(27.5%) in hen meat and 38(31.6%) in eggshell. In egg-shell samples*: C. jejuni* 20, *C. coli* 14, *C. lari* 3 and *C. concisus* 1 case. In meat common Penner serotyping for *C. jejuni* O_2 had the highest rate. In hen, common Penner serotyping: for *C. jejuni* O_3 and in egg-shell for O_1, O_2 and O_3 had the highest rate.

Conclusion: Most infection of campylobacter was found in egg-shell; most common species in these three samples were *C. jejuni*, then *C. coli* and *C. lari*. No *C. consicus* was found in meat but it was found in hen and egg-shells. In common Penner serotyping for *C. jejuni* O_2 and O_3 were the most common and for *C. coli* in meat O_{49} and in hen and eggshell O_5 were the highest.

Keywords: Campylobacter, Serotyping, *C. jejuni*, *C. coli*

1. Background

Campylobacters are curved rods that were classified as vibrios for many years. *Campylobacter* spp. Have been are microaerophilic, thermotolerating Gram negative bacteria. Use of oxygen-quenching agents, a microaerobic atmosphere, and antibiotics that suppress competitors, significantly improve *Campylobacter* survival. Furthermore, *Campylobacter* spp. are very sensitive to freezing and can die at room temperature as well. For investigation, the sample analysis should be initiated as soon as possible after it is received in the laboratory. Only samples received within the temperature range of 0-15°C should be analyzed (1, 2).

Campylobacters are found to be associated with animal and human diseases. The most common human disease caused by *Campylobacters* is acute gastroenteritis. Infection occurs in infants, elderly people, and patients with underlying disease. Fever, bloody diarrhea, headache and abdominal pain are observed in this disease. Campylobacteriosis is caused by members of these bacteria. Campylobacteriosis is a self-limited disease and antibiotic treatment is not generally suggested. Nevertheless, antimicrobial therapy in early phase of infection may cause to decrease the symptoms of this disease. Therm-otolerant *Campylobacteria* such as *C. jejuni* and *C. coli* are the most important pathogenic strains. Slaughtering may lead to direct contamination. Contamination can also occur directly through air, bird to bird, via equipment and water. Among the foods that carry these microorganisms, the chicken meat is most common (3, 4).

Campylobacter jejuni is detected in intestinal content of poulty. Furthermore, infection with *C. jejuni* has been identified as the most important predisposing factor for the development of the neurological disorder Guillain–Barre´syndrome (GBS). Structural studies of lipopolysaccharide (LPS) extracted from *C. jejuni* have shown that the terminal regions of the LPS core oligosaccharide (OS) of specific serotypes mimic the structures of human gangliosides, particularly in strains associated with GBS development (5) .

Direct observation is the best method for infection diagnosis. Studying bacterial movement in phase-contrast microscopy could be useful. Cary-Blair is a good transportation medium and two most useful media for growing and isolating bacteria are Campy-CVA and Campy-Bap. *Campylobacter* could pass from filters with diameters 0.56 and 0. 45 micrometer and it can be used as a clinical method for isolation. These bacteria grow in 37-42°C and with supplement of 3-15% O_2 or 3-5% CO_2. These are some methods for *Campylobacter* typing such as serotyping, biotyping, ribotyping, phagetyping and exotyping. Among these methods, serotyping is the best way for typing with high isolation. Serotyping with Penner scheme is based on O-antigen of LPS and it is heat-labile. Serotyping with Lior scheme is based on labile antigen and more time consuming and expensive. Based on Penner scheme, *C. jejuni* and *C. coli* have 65 serotypes. *C. coli* is common in birds and dogs. Biochemical scheme of *C. coli* is like *C. jejuni*, however, *C. coli* cannot hydrolyze hippurate. With consideration of high prevalence of *Campylobacter*in red-meat, chicken- meat and egg-shell, we investigated a suitable method for determining their prevalence, the most common species and serotyping group (6, 7).

2. Objectives

In this study we attempted to find a better way for laboratory diagnosis of *Campylobacter*strains in red-meat, chicken-meat and egg-shell and their serotypes in 330 samples in Zanjan city.

3. Materials and Methods

This article describes the prevalence of *Campylobacter* infection, its strains and common serotyping group in 330 samples of red- meat, chicken-meat and eggshells. First samples from selective cases were prepared and then with following three methods; enrichment, selective Preston & Skirrow media and filteration with membrane filters; *Campylobacte r* were insolated in agar plate. Bacterial species were identified with physiological and biochemical tests. Penner serotyping was used with reference antiserum Ag-O and direct agglutination (8).

3.1. Red- and chicken-meat

Twenty five grams of meat was used for this study. After incubation in lactose-broth for 24h, 1ml of samples shift to Selenite-F and 10ml to bismute-sulfite agar and after 24h incubation in 37ºC for chicken-meat and 42ºC for red-meat, biochemical tests and serotyping were done. Preston broth agar was also used as a media for meat. When suspected colonies were detected, confirming tests including Gram stain, grownat 25°C, oxidase and catalase tests, sensitivity to nalidixic acid and cephalothin and hippurate hydrolysis were performed.

3.2. Egg-shell

The eggs were collected from hens with cloacal swab positive for *Campylobacter* spp.. After maceration the shell of eggs *Campylobacter* spp.can be detected. After 12 hours at room temperature, the disinfected and non-disinfected eggs were broken. 10 g of the macerated shells were seeded in 200 ml of Bolton broth and incubated at 37ºC during 24 hours in a microaerobic atmosphere. The isolation and identification procedures were performed as same as those used for meat (9-11).

The characteristic colony-forming units that appeared on the plates were confirmed as *Campylobacter* spp. by gram staining and under phase-contrast microscopy for typical movement and morphology and other biochemical tests including growth at 25°C, oxidase and catalase tests, sensitivity to antibiotics which was referred before. Penner scheme was done with references antiserum Ag-O with direct agglutination method for bacterial strains isolated with physiological and biochemical tests. Campy-thio was used as the transportation media. In enrichment method, samples were inoculated to Preston enrichment broth and incubated at 24ºC for 24h and then Skirrow-Preston was used as selective media for about 48h. In filtration samples transportation medium, diluted in normal saline was poured on 0.65 micrometer filters in Brucella-agar and incubated for 48h. To prepare Campy-thio medium, Thioglycolate broth, vancomycin, trimethoperim, polymyxin, and amphotericin were used. To prepare Preston enrichment broth, nutrient broth, defibrinated sterile blood of horse with saponin and cycloheximide, trimethoperim, rifampicin and polymyxinwere used. In selective media(Skirrow agar), there was nutrient agar, defibrinated sterile blood of horse with saponin, vancomycin, trimethoperim and polymyxin as in Preston agar (12).

To determine bacterial species, biochemical tests were used. The confirmation of the identity of isolates was based on characteristic reactions for hippurate hydrolysis, indoxyl- acetate hydrolysis and urease activity. Other characters such as growth temperature, catalase and oxidase test, nitrate reduction, growth in 1% glycin and 3/3% NaCl, producing H_2S and TTC-sensitive were used to identify bacterial species (13).

For serotyping Penner scheme AgO with direct agglutination was used. Microbial suspension of overnight culture was produced with addition of 1ml PBS and incubated in 100ºC, and then 5ml PBS was added again. In microtitre plate we used 25 microlitre of suspension and 25 microlitre of 1/10 dilution of each antiserum. After 150 minutes shaking in room temperature, plates were examined for agglutination test. If no agglutination was observed, additional time (60 minutes) was given to examine the plates again. At this time if no agglutination was observed, it was considered true negative. To calculate significant difference between methods to isolate bacterium, strains and serotyping in diseases, we used chi-square test in SPSS- software. A value of $p<0.05$ was considered statistically significant (14, 15).

4. Results

The prevalence of *Campylobacter* was 21(23%) in red-meat, 33(27.5%) in chicken-meat and 38(31.6%) in egg-shell. Strains in food items: in red-meat: 13 *C.jejuni*, 6 *C.coli* and 2 *C.lari*. In chicken-meat: 22 *C.jejuni*, 9 *C.coli*, 1 *C.lari* and 1 *C.concisus* (Table 1). In egg-shell: 20 *C.jejuni*, 14 *C.coli*, 3 *C.lari* and 1 *C.concisus* (Table2). Penner-serotyping showed these results for red-meat: in *C.jejuni*: O_2= 4, O_3= 4, O_{21}= 3 and O_{18}= 2 and in C.*coli*: O_{49}=3, O_{25}=1 and O_{30}=1, for chicken-meat: in *C.jejuni*: O_3= 5, O_1= 4, O_2= 5, O_{13}= 3, O_{50}= 3, O_{18}= 1, O_{14}= 1 and in *C.coli*: O_5= 4, O_{49}= 3, O_{48}= 1 and O_{56}= 1 and for egg-shell: in *C.jejuni*: O_1, O_2 and O_3= 4, O_7, O_{14} and O_{13}= 2, O_{44} and O_{18}= 1 and in *C.coli*: O_{48} and O_{49}= 2, O_{28} = 3, O_{25}= 1, O_5= 6(Table3).

Table 1. Prevalence of *Campylobacter* spp.

Type of sample	Red-meat	Chicken-meat	Egg-shell
No. of contaminated samples	21	33	38
Total number	90	120	120

Table 2. Foundedstrains in samples

Types \ Strains	*C. jejuni*	*C. coli*	*C. lari*	*C. concisus*
Red-meat	13	6	2	-
Chicken-meat	22	9	1	1
Egg-shell	20	14	3	1

Table 3. Serotyping in red-meat, chicken-meat and egg-shell

Red-meat/chicken-meat/egg-shell	Serotype			Frequency			Percent of frequency		
C. coli	O_{49}	O_5	O_5	3	4	6	50	44.44	42.85
	O_{25}	O_{48}	O_{25}	2	1	1	33.33	11.11	7.14
	O_{30}	O_{49}	O_{28}	1	3	3	16.66	33.33	21.42
C. jejuni	O_2	O_{56}	O_{48}	4	1	2	30.76	11.11	14.28
	O_3	O_1	O_{49}	4	4	2	30.76	18.18	14.28
	O_{21}	O_2	O_1	3	5	4	23.07	27.27	20
	O_{18}	O_3	O_2	2	5	4	15.38	27.27	20
	-	-	O_3	-	3	4	-	13.63	20
	-	-	O_7	-	-	2	-	-	10
	-	-	O_{13}	-	-	2	-	-	10
	-	-	O_{14}	-	-	2	-	-	10
	-	-	O_{18}	-	-	2	-	-	10
	-	-	O_{44}	-	-	1	-	-	5

5. Discussion

Campylobacter jejuni has been identified as one of the main causes of food poisoning. The most common species of *Campylobacter* in animals and foods are *C.jejuni, C. coli* and *C. lari*. Among these, *C. jejuni* is the most commonly involved pathogen in human gastroenteritis. Hippurate hydrolysis is the only biochemical test which can differrentiate between *C. jejuni* and other *Campylobacter* species. Campylobacteriosis is one of the most common foodborne infections in the U.S. and it has been estimated that annually 1% of the U.S. population can be infected by this disease. Campylobacter infections symptoms vary from diarrhea, cramping, abdominal pain and fever, to hyperplasia and hypertrophy, have the most common food borne route via poultry meat (16).

Alimentary tract of wild and domesticated birds and mammals, mainly chicken and turkey contain high numbers of *C. jejuni*. Serotyping belongs to the most widespread phenotyping methods. Since no research had been done in Zanjan city on the infection of *Campylobacter* disease and with regard to importance of these bacteria in gastroenteritidis infection, it is necessary to know the exact rate of prevalence of these bacteria in human infection disease, their strains and serotyping. On the other hand, since these bacteria infect from red and bird-meat to human, the source of bacterial prevalence with serotyping of *Campylobacter* infection in society as a whole will be determined leading to avenues for prevention. High infection of *Campylobacter* had been foundin egg-shell, and the most common species found in these samples were *C. jejuni*, and then *C. coli* and *C. lari*. No *C.consicus* was found in red-meat, however, it was found in hen-meat and egg-shells. These high rates of infection in many countries lead to financial burden for the treatment. In epidemiological study, determination of bacterial strains was not enough and it is necessary to do serotyping, biotyping, phagetyping and ribotyping to precisely determine microorganisms. Serotyping is based on Ag-O in *Campylobacters* named Penner serotyping and it is simple and useful method. The Penner serotyping scheme uses 62 antisera (HS) at present, and the Lior's system works with 122 antisera (HL). Only a selected group of antisera that matches with *Campylobacter* spp. isolated from Iran is commonly used. Usually, for Penner serotyping of *C. jejuni*, O_2 and O_3 are used and for *C. coli* in meat O_{49} and in hen-meat and egg-shell O_5 are commonly used (17).

To prevent this bacterial infection, it is recommended to use sterilized water in birds' feed and to perform slaughtering, skinning and evisceration under aseptic conditions. Ingestion of raw milk and unchlorinated water should also be avoided (13).

6. Conclusion

The current study showed the prevalence of presence of *Campylobacter* in human food, and therefore a way to prevent campylobacteriosis. It is recommended to use chlorinated water in birds' feed and to perform slaughtering, skinning and evisceration under aseptic conditions. Before the consumption of foods, the temperature at the center of chicken breasts and chicken thighs must reach at least 77°C and 82°C respectively. Ingestion of raw milk and unchlorinated water should also be avoided (13)

Conflict of Interests

The authors declare they have no conflict of interests.

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Authors' Contributions

Shiva modirrousta: Study conception and design, Reza Shapouri: Analysis and interpretation of data, Sama Rezasoltani: drafting of the manuscript, Hamed Molaabaszade: critical revision

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