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* were incubated. Bacterial species were identified with physiological and biochemical tests. Penner serotyping was defined with reference antisera 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* O2 had the highest rate. In hen, common Penner serotyping: for *C. jejuni* O3 and in egg-shell for O3, O8 and O6 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. concisus* was found in meat but it was found in hen and egg-shells. In common Penner serotyping for *C. jejuni* O2 and O3 were the most common and for *C. coli* in meat O8 and in hen and eggshell O8 were the highest.

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

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1. **Background**

*Campylobacter* are curved rods that have been classified as vibrios for many years. They are microaerophilic, thermotolerating Gram negative bacteria. Oxygen-quenching agents, microaerobic atmosphere, and antibiotics that suppress competitors, are used to significantly improve *Campylobacter* growth and 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 associated with animal and human diseases. Acute gastroenteritis which occurs in infants, elderly people, and patients with underlying disease with fever, bloody diarrhea, headache and abdominal pain symptoms is the most common human disease caused by *Campylobacters*. Campylobacteriosis is caused by members of these bacteria. Campylobacteriosis is a self-limited disease and antibiotic treatment is not usually recommended. Nevertheless, antimicrobial therapy in early phase of infection may cause to decrease the symptoms of this disease. Thermotolerant Campylobacteria such as *C. jejuni* and *C. coli* are the most important pathogenic strains. Slaughtering may result in direct contamination of specimens. Other ways of contamination are directly via equipment, water, air and even bird to bird. Foods can carry these microorganisms too. Chicken meat is most common food to carry them (3, 4).

*Campylobacter jejuni* is detected in intestinal content of poultry. Furthermore, infection with *C. jejuni* has been proven to be as the main predisposing factor for the development of Guillain–Barre syndrome (GBS) which is a neurological disorder. Structural studies of lipopolysaccharide (LPS) extracted from *C. jejuni* have shown that LPS core oligosaccharide (OS) of specific serotypes is similar to the structures of human gangliosides, specifically in strains related to GBS syndrome(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% O2 or 3-5% CO2. These are some methods for *Campylobacter* typing such as serotyping, biotyping, ribotyping, phageotyping 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 filtration with membrane filters; *Campylobacte r* were isolated in agar plate. Bacterial species were identified with physiological and biochemical tests. Penner serotyping was used with reference antisera 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 Selene-F and 10ml to bismuth-sulfite agar and after 24h incubation in 37°C for chicken-meat and 42°C 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, grown 25°C, oxidase and catalase tests, sensitivity to nalidixic acid and cephalothin 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 disinfectated and non-disinfectated eggs were broken and 10 g of the macerated shells were seeded in 200 ml of Bolton broth and incubated at 37°C for 24 hours in a microaerobic atmosphere. The isolation and identification procedures were performed the same way it was done for meat (9-11).

It was confirmed that The characteristic colony-forming units that appeared on the plates were *Campylobacter* spp. by gram staining and phase-contrast microscopy for typical movement and morphology. Other biochemical tests including growth at 25°C, oxidase and catalase tests, sensitivity to antibiotics also confirmed the identity of isolates. Penner scheme was done with references antisera 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 24°C 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, trimethoprim, polymixin, and amphotericin were used. To prepare Preston enrichment broth, nutrient broth, defibrinated sterile blood of horse with saponin and cycloheximide, trimethoprim, rifampicin and polymixin were used. In selective media(Skirrow agar), there was nutrient agar, defibrinated sterile blood of horse with saponin, vancomycin, trimethoprim and polymyxin as in Preston agar (12).

Biocmical tests were used To determine bacterial species, purpurrhydrate hydrolysis, indoxyl- aceta hydrolysis and urease activity were used to confirm the identity of the isolates. Other characters such as growth temperature, catalase and oxidase test, nitrate reduction, growth in 1% glycine and 3/3% NaCl producing H₂S 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 100°C, and then 5ml PBS was added again. In microtirte plate we used 25 microlitre of suspension and 25 microlitre of 1/10 dilution of each antisera. 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 *Clari* In chicken-meat: 22 *C.jejuni*, 9 *C.coli*, 1 *Clari* and 1 *C.concisus* (Table 1). In egg-shell: 20 *C.jejuni*, 14 *C.coli*, 3 *Clari* and 1 *C.concisus* (Table 2). Penner-serotyping showed these results for red-meat: in *C.jejuni*: O₁= 4, O₂= 4, O₃= 3 and O₄= 2 and in *C.coli*: O₁=3, O₂=1 and O₃=6, for chicken-meat: in *C.jejuni*: O₁= 5, O₂= 4, O₃= 5, O₄= 3, O₅= 3, O₆= 1, O₇= 1 and in *C.coli*: O₁= 4, O₂= 3, O₃= 1 and O₄= 1 and for egg-shell: in *C.jejuni*: O₁, O₂ and O₃= 4, O₄, O₅ and O₆= 2, O₇ and O₈= 1 and in *C.coli*: O₁= 3, O₂= 3, O₃= 1, O₄= 0 (Table3).

**Table 1. Prevalence of Campylobacter spp.**

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Red-meat</th>
<th>Chicken-meat</th>
<th>Egg-shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of contaminated samples</td>
<td>21</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>Total number</td>
<td>90</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

**Table 2. Foundedstrains in samples**

<table>
<thead>
<tr>
<th>Types</th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>C. lari</th>
<th>C. concisus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-meat</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Chicken-meat</td>
<td>22</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Egg-shell</td>
<td>20</td>
<td>14</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Red-meat/chicken-meat/egg-shell</th>
<th>C. jejuni</th>
<th>Frequency</th>
<th>Percent of frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₁</td>
<td>O₂</td>
<td>O₃</td>
<td>O₄</td>
</tr>
<tr>
<td>O₂</td>
<td>O₃</td>
<td>O₄</td>
<td>O₅</td>
</tr>
<tr>
<td>O₃</td>
<td>O₄</td>
<td>O₅</td>
<td>4</td>
</tr>
<tr>
<td>O₄</td>
<td>O₅</td>
<td>O₆</td>
<td>4</td>
</tr>
<tr>
<td>O₅</td>
<td>O₆</td>
<td>O₇</td>
<td>3</td>
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<tr>
<td>O₆</td>
<td>O₇</td>
<td>O₈</td>
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</tr>
<tr>
<td>O₇</td>
<td>O₈</td>
<td>O₉</td>
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<tr>
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<td>O₉</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O₉</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>O₁₀</td>
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<td>-</td>
<td>2</td>
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<tr>
<td>O₁₁</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>O₁₂</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>
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 differentiate 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 have the mostly derive from the poultry meat with symptoms varying from diarrhea, cramping, abdominal pain and fever, to hyperplasia and hyper-trophy, 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 gastroenteritis 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 found in egg-shell, and the most common species found in these samples were C. jejuni, and then C. coli and C. lari. No C. concisus 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 group of Penner serotyping scheme uses 62 antisera (HS) at present, and the

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 hygienic water in feeding birds and to carry out all slaughtering steps 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 Rezassoltani: drafting of the manuscript, Hamed Molaabasazade: critical revision

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