Prevalence and Antibiotic Susceptibility of Campylobacter species Isolated From Chicken and Beef Meat

Hossein Dabiri 1,2; Shadi Aghamohammadi 1; Hossein Goudarzi 1; Maryam Noori 1; Manouchehr Ahmadi Hedayati 1; Seyed Mehdi Ghoreyshiamiri 1

1Department of Medical Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran
2Corresponding author: Hossein Dabiri, Department of Medical Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

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Background: To study prevalence of Campylobacter spp. in chicken and beef meat, and determine the drug susceptibility of strains, 450 samples in Tehran, Iran were investigated.

Objectives: This study aimed to determine the prevalence and the antimicrobial resistance of entropathogenic Campylobacter strains, especially C. jejuni isolated from raw chicken and beef meat in Tehran-Iran.

Materials and Methods: Out of 250 chickens and 200 beef meats, 121(26.8 %) contaminated cases with Campylobacter strains were isolated. Campylobacter was isolated from a significantly larger number of chickens (44%) than beef meats (5.5%) [P < 0.05].

Results: From all isolated Campylobacter organisms, 91 (76.8%) species were identified as C. jejuni and 28 cases (23.1%) as C. coli. Susceptibilities of 121 strains (93 C. jejuni and 28 C. coli) were determined against 12 antimicrobial drugs using the disk agar diffusion method. Resistance to nalidixic acid (75%) and ciprofloxacin (50%) was an alarming finding, moreover, 32.6% of isolates was resistant to tetracycline, 10.8% to ampicillin, 29.3% to colistin and 26.1% to amoxicillin. The highest sensitivity was seen to erythromycin (95%) and gentamicin (96%).

Conclusions: These results showed that a high proportion of chicken and beef meat in Iran is contaminated with Campylobacter, particularly with Campylobacter jejuni. The high rate of contamination, especially chicken is a significant public health concern. Most of the isolates were resistant; therefore, human infection with Campylobacter spp via consumption of these products is possible.

Keywords: Campylobacter; Anti-Bacterial Agents; Chickens; Beef Meat; Iran

1. Background

Campylobacter is one of the most common causes of acute bacterial gastroenteritis in human worldwide (1). Campylobacteriosis is a zoonotic disease; domestic animals such as poultry, pigs, and cattle may act as reservoirs for Campylobacter spp. (2). The consumption of chicken and chicken products, are implicated in higher number of worldwide outbreaks of acute Campylobacter enterocolitis in both industrialized and developing countries, especially among children, the elderly and immuno-suppressed patients over the recent years (3, 4).

Besides, low infection dose of C. jejuni, its rate of infection increases along with the ingested dose (5). C. jejuni and C. coli are isolated mostly from humans. C. jejuni is often implicated as the cause of campylobacteriosis, while C. coli seems to be less frequent in causing human acute diarrhea (6). Recently, food-borne infections with resistant pathogens have emerged as a threat to human health. The concern for this food-borne infection has increased because of the frequent isolation of antimicrobial resistant Campylobacter strains in humans and animals (7, 8).

This is almost a consequence of the massive use of antibiotics in modern intensive animal and poultry production units for therapy and prevention of their diseases (9). Illness caused by Campylobacter is usually self-limiting and therapy is not required, except in severe episodes of disease or in immunocompromised patients, that antibiotic therapy may be necessary. In these specific cases, therapy may be complicated by the fact that antimicrobial resistance in Campylobacter isolates from human infections has become increasingly common (10).

According to different reports from Iran, the prevalence of Campylobacter in clinical samples ranges from 4 to 10 percent (11-13). However, there is not any information about prevalence and antibiotic susceptibility of different species of Campylobacter isolated from food samples in Iran.

Implication for health policy/practice/research/medical education: Campylobacter is known as a major cause of food-borne diseases worldwide, particularly in developing countries like Iran where high prevalence of this bacterium has been reported. Chicken and beef meat are the main sources of human infections. In many cases, treatment of Campylobacter infections is based on empirical treatment. Prior knowledge of Campylobacter infections and their antimicrobial susceptibility profile are crucial for effective and cost-benefit treatment. Therefore, we aimed to study Campylobacter prevalence and its antibiotic susceptibility profile.
2. Objectives
This study aimed to determine the prevalence and the antimicrobial resistance pattern of entropathogenic Campylobacter strains, especially C. jejuni isolated from raw chicken and beef meat in Tehran-Iran.

3. Material and Methods

3.1. Samples
To investigate the prevalence of Campylobacter, a total of 450 samples, including chicken (n = 250) and beef meat (n = 200) were collected from Tehran, from April 2011 to May 2012. All samples were received by the laboratory of the Department of Microbiology, and microbiological analysis was carried out within 2 hours after the collection.

3.2. Isolation and Identification of Campylobacter spp.
To isolate Campylobacter, 25 g of each sample after hemogenizing was pre-enriched in Campylobacter enrichment broth base (Preston enrichment broth base, HIMEDIA, Mumbai, India, M899) supplemented with Campylobacter selective supplement IV (HIMEDIA, Mumbai, India, FD58) and 5% defibrinated sheep blood. After inoculation at 42°C for 24 h in a microaerophilic condition, 0.1 mL of the sample was streaked onto Campylobacter selective agar base (HIMEDIA, Mumbai, India, M994) supplemented with an antibiotic supplement for the selective isolation of Campylobacter species (Campylobacter Supplement-2, Blaser Wang, HIMEDIA, Mumbai, India, FD 006) and 5% (V/V) defibrinated sheep blood and incubated for 48 h at 42°C under the same condition. One presumptive Campylobacter colony from each selective agar plate was subcultured and tested for Gram staining, production of catalase, oxidase and hippurate hydrolysis. One strain from each Campylobacter-positive sample was selected for susceptibility testing.

3.3. Antimicrobial Susceptibility Testing
Susceptibilities of 121 strains (93 C. jejuni and 28 C. coli) were determined against 12 antimicrobial drugs using the disk agar diffusion method. The test was carried out according to NCCLS (National Committee on Clinical Laboratory Standards) protocols. The antimicrobial agents that tested were as follows; nalidixic acid (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), tetracycline (15 µg), streptomycin (30 µg), gentamicin (10 µg), amoxicillin (30 µg), chloramphenicol (30 µg), amoxicillin (30 µg), spectinomycin (100 µg), colistin (10 µg) and neomycin (30 µg). Zone diameters were measured, recorded, and interpreted in accordance with NCCLS guidelines (14).

3.4. Statistical Analysis
Statistical analysis of results was performed with SPSS/PC 11.5 software (SPSS, Chicago, IL). The chi-square test and Fisher’s exact two-tailed test were used for statistical analysis. A P value less than 0.05 was considered statistically significant.

4. Results
Out of 450 samples, 250 chickens and 200 beef meats, 121 (26.8%) isolates were detected as Campylobacter spp. based on biochemical and microbiological tests. Of these isolates, 93 (76.8%) species were identified as C. jejuni and 28 (23.1%) as C. coli. Campylobacter was isolated from a significantly larger number of chickens 110 (44%) compare to beef meats 11 (5.5%) (P < 0.05). Of Campylobacter strains isolated from chicken, 87 (79%) and 23 (21%) were identified as C. jejuni and C. coli respectively. In case of isolates from beef meats, the prevalence of C. jejuni and C. coli were 6 (54.5%) and 5 (45.5%) respectively. The association between type of the Campylobacter and chicken and beef meats was not statistically significant (P= 0.1). Antibiotic susceptibility test against 12 antimicrobial agents was done for 121 isolates (93 C. jejuni and 28 C. coli) (Table 1). Fifty-one (42.1%) isolates were resistant to at least three antibiotics. Regardless of the type of Campylobacter, the highest rate of resistance (75%) was seen against nalidixic acid. While gentamycin was the most active (96%) antibiotic against studied isolates followed by chloramphenicol (95%), erythromycin (95%), spectinomycin (94%) and streptomycin (94%).

5. Discussion
Awareness of the public health implications of Campylobacter infections has evolved for over a century (15). Campylobacteriosis is a leading cause of gastroenteritis in many countries, and it has been isolated from a considerable number of patient with gastroenteritis in Iran (3-5, 11-13). Prevalence of Campylobacter spp. in Iran is lower than developed countries (1, 16, 17). However, similar finding was reported by Dadi et al. (18). According to our results, Campylobacter was recovered at higher prevalence in chicken samples than beef meat and C. jejuni was the most predominant Campylobacter species recovered from meat and chicken. These data are in accordance with reports from other countries (18-21). Proportion of C. jejuni and C. coli in chicken and beef meat samples was similar. C. jejuni was significantly more prevalent than C. coli in both chicken and beef meat (P < 0.05).

The increasing rate of human infections caused by antimicrobial-resistant strains of C. jejuni makes clinical management of cases with campylobacteriosis more difficult (12, 22, 23). Antimicrobial resistance can prolong illness and compromise treatment of patients with bacteremia. The rate of antimicrobial-resistant enteric infections is highest in the developing countries, where the use of antimicrobial drugs in humans and animals is relatively unrestricted (20).
Resistance of C. jejuni to quinolones was the most alarming finding in this study. Interestingly, in a recently published study from Iran, clinical isolates of Campylobacter spp. showed high resistance to same antibiotics (12), which may be as a result of consumption of contaminated food that harbors antibiotic resistant Campylobacter. Thus, administration of quinolones as the drug of choice for acute diarrhea in Iran seems to be recommended. The recent study has employed larger samples and ranges of antimicrobial agents along with 11 antimicrobials among Campylobacter coli isolated from pigs on 80 grower-finisher farms in Ontario. Can J Vet Res. 2007;71(3):189-94.

In conclusion, the result showed that a high proportion of chicken and beef meat in Iran is contaminated with Campylobacter, particularly with Campylobacter jejuni. The high rate of contamination in chicken meat alarm a significant public health concern. Most of the isolates were resistant; therefore, there is a possible risk of human infection with Campylobacter spp. via consumption of these products.

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Authors’ Contribution
All authors had participated in the study.

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References

Table 1. Number and Percentages of Antimicrobial Resistant Campylobacter Strains Isolated From Chicken and Beef Meat a

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>C. jejuni (n = 93)</th>
<th>C. coli (n = 28)</th>
<th>Total (n = 121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>26 (28.5)</td>
<td>05 (18.1)</td>
<td>31 (26.1)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>12 (12.7)</td>
<td>01 (4.5)</td>
<td>13 (10.8)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>04 (4.2)</td>
<td>01 (4.5)</td>
<td>05 (4.3)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>48 (51.4)</td>
<td>13 (45.4)</td>
<td>61 (50)</td>
</tr>
<tr>
<td>Colistin</td>
<td>32 (34.2)</td>
<td>04 (13.6)</td>
<td>36 (29.3)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>04 (4.2)</td>
<td>01 (4.5)</td>
<td>05 (4.3)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>03 (2.8)</td>
<td>01 (4.5)</td>
<td>04 (3.2)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>66 (71.4)</td>
<td>24 (86.3)</td>
<td>90 (75)</td>
</tr>
<tr>
<td>Neomycin</td>
<td>08 (8.5)</td>
<td>02 (9)</td>
<td>10 (8.6)</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>04 (4.2)</td>
<td>02 (9)</td>
<td>06 (5.4)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>05 (5.7)</td>
<td>01 (4.5)</td>
<td>06 (5.4)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>33 (35.7)</td>
<td>06 (22.7)</td>
<td>39 (32.6)</td>
</tr>
</tbody>
</table>

a Data are presented as No. (%)