Int J Enteric Pathog. 2014 May; 2(2): e17087. DOI: 10.17795/ijep17087

Published online 2014 May 10. **Research Article**

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

Hossein Dabiri [1,*]; Shadi Aghamohammad [1]; Hossein Goudarzi [1]; Maryam Noori [1]; Manouchehr Ahmadi Hedayati [1]; Seyed Mehdi Ghoreyshiamiri [1]

[1]Department of Medical Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

[*]*Corresponding author*: Hossein Dabiri, Department of Medical Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran . Tel: +98-9122839824, Fax: +98-9122839824, E-mail: hodabiri@gmail.com

Received: December 24, 2013; **Revised:** February 3, 2014; **Accepted:** February 24, 2014

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, 93 (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 colisitin 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 imperial treatment. Prior knowledge of *Campylobacter* infections and theirntibiotic 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, FD158) 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), colisitin (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* starins 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 *Campylobacte*r 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).

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

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

[a] Data are presented as No. (%)

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 revised. Current study has employed larger samples and not restricted to a special season. Remarkable difference between antibiotic susceptibility pattern of *C. jejuni* and *C. coli* has been reported. The incidence of resistance to most of the tested antimicrobial agent in this study was generally higher for *C. jejuni* than for *C. coli* (Table 1). This finding confirmed what has been previously described (8, 24), although results from another study demonstrated that *C. coli* was generally more resistant than *C. jejuni* (9, 18, 23, 25). Owing to the increased reporting of antimicrobial resistance in *Campylobacter* worldwide, attempts should be made to control their use in animal husbandry.

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 alarms 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.

Acknowledgements

There is no acknowledgement.

Authors' Contribution

All authors had participated in the study.

Funding/support

The study is self-funded.

Financial Disclosure

There is no financial disclosure.

References

1. Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA. Prevalence of Arcobacter and Campylobacter on broiler carcasses during processing. *Int J Food Microbiol.* 2007;**113**(1):16–22.
2. Ishihara K, Kira T, Ogikubo K, Morioka A, Kojima A, Kijima-Tanaka M, et al. Antimicrobial susceptibilities of Campylobacter isolated from food-producing animals on farms (1999-2001): results from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Int J Antimicrob Agents.* 2004;**24**(3):261–7.
3. *CDC (The US Centers for Disease Control and Prevention), 2005. Campylobacter infection.*. Available from www.cdc.gov/ncidod/dbmd/diseaseinfo/campylobacter_g.htm..
4. Corry JEL, Atabay HI. Poultry as a source of Campylobacter and related organisms. *Journal of Applied Microbiology.* 2001;**90**(S6):96S–114S.
5. Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ. Experimental Campylobacter jejuni infection in humans. *J Infect Dis.* 1988;**157**(3):472–9.
6. Skirrow MB. Campylobacteriosis. In: Palmer SR, Soulsby L, Simpson DIH editors. *Zoonoses biology clinical practice and public health control.*: Oxford Medical Publications; 1998. pp. 37–46.
7. Varela NP, Friendship R, Dewey C. Prevalence of resistance to 11 antimicrobials among Campylobacter coill isolated from pigs on 80 grower-finisher farms in Ontario. *Can J Vet Res.* 2007;**71**(3):189–94.
8. Saenz Y, Zarazaga M, Lantero M, Gastanares MJ, Baquero F, Torres C. Antibiotic resistance in Campylobacter strains isolated from animals, foods, and humans in Spain in 1997-1998. *Antimicrob Agents Chemother.* 2000;**44**(2):267–71.
9. Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M, Perin R. Occurrence and resistance to antibiotics of Campylobacter jejuni and Campylobacter coli in animals and meat in northeastern Italy. *Int J Food Microbiol.* 2003;**82**(3):281–7.
10. Engberg J, Neimann J, Nielsen EM, Aerestrup FM, Fussing V. Quinolone-resistant Campylobacter infections: risk factors and clinical consequences. *Emerg Infect Dis.* 2004;**10**(6):1056–63.

11. Soltan Dallal M, Bokaeiyan M. Study of Campylobacter diarrhea in Zahedan region, Iran. *Iran J Public Health.* 1995;**24**(3-4):45–54.
12. Feizabadi MM, Dolatabadi S, Zali MR. Isolation and drug-resistant patterns of Campylobacter strains cultured from diarrheic children in Tehran. *Jpn J Infect Dis.* 2007;**60**(4):217–9.
13. Hassanzadeh P, Motamedifar M. Occurrence of Campylobacter jejuni in Shiraz, Southwest Iran. *Med Princ Pract.* 2007;**16**(1):59–62.
14. National Committee on Clinical Laboratory Standards.. *Performance standards for antimicrobial susceptibility testing-elevent information supplement, M100-S11.*PA, USA: NCCLS Villanova; 2001.
15. The historical background of Campylobacter infection: new aspects. In: Kist M editor. . *3rd International Workshop on Campylobacter Infections, Public Health Laboratory Service.* 1985 pp. 23–7.
16. Harrison WA, Griffith CJ, Tennant D, Peters AC. Incidence of Campylobacter and Salmonella isolated from retail chicken and associated packaging in South Wales. *Lett Appl Microbiol.* 2001;**33**(6):450–4.
17. Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, et al. Prevalence of Campylobacter spp., Escherichia coli, and Salmonella serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Appl Environ Microbiol.* 2001;**67**(12):5431–6.
18. Dadi L, Asrat D. Prevalence and antimicrobial susceptibility profiles of thermotolerant Campylobacter strains in retail raw meat products in Ethiopia. *Ethiop J Health Dev.* 2008;**22**(2):195–200.
19. Granic K, Krcar D, Uhitil S, Jaksic S. Determination of Campylobacter spp. in poultry slaughterhouses and poultry meat. *Veterinarski Archiv.* 2009;**79**(5):491–7.
20. Altekruse SF, Stern NJ, Fields PI, Swerdlow DL. Campylobacter jejuni–an emerging foodborne pathogen. *Emerg Infect Dis.* 1999;**5**(1):28–35.
21. Mayrhofer S, Paulsen P, Smulders FJ, Hilbert F. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *Int J Food Microbiol.* 2004;**97**(1):23–9.
22. Snelling WJ, Matsuda M, Moore JE, Dooley JS. Campylobacter jejuni. *Lett Appl Microbiol.* 2005;**41**(4):297–302.
23. Sallam KI. Prevalence of Campylobacter in chicken and chicken by-products retailed in Sapporo area, Hokkaido, Japan. *Food Control.* 2007;**18**(9):1113–20.
24. Aarestrup FM, Engberg J. Antimicrobial resistance of thermophilic Campylobacter. *Vet Res.* 2001;**32**(3-4):311–21.
25. Avrain L, Humbert F, L'Hospitalier R, Sanders P, Vernozy-Rozand C, Kempf I. Antimicrobial resistance in Campylobacter from broilers: association with production type and antimicrobial use. *Vet Microbiol.* 2003;**96**(3):267–76.