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Detection of multi-antibiotic resistant *Campylobacter coli* and *Campylobacter jejuni* in beef, mutton, chicken and water buffalo meat in Ahvaz, Iran

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Abstract

Campylobacter jejuni and *C. coli* are the main causes of gastrointestinal diseases in humans even in industrialized countries affecting public health. The aim of the current study was to evaluate the occurrence and antibiotic resistance of *C. jejuni* and *C. coli* in chicken meat, beef, mutton and water buffalo meat slaughtered in Ahvaz city, Iran. A total of 380 samples including chicken meat from industrial abattoirs (n = 150), chicken meat from traditional abattoirs (n = 50), fresh packed chicken meat from local markets (n = 30) and beef, mutton and water buffalo meat from industrial abattoirs (50 samples for each meat) in Ahvaz,were collected and tested for the presence of *Campylobacter* spp. The procedure was one-step enrichment in Preston enrichment broth followed by plating on supplemented blood agar for 24 hr under microaerophilic conditions at 42 ˚C. Suspected colonies were tested by polymerase chain reaction assay and susceptibility of the confirmed isolates to various antibiotics was investigated by the Kirby-Bauer disk diffusion method. Overall, 32 samples (8.40%) were contaminated with *Campylobacter* spp. Mutton was the most contaminated meat (24%), while fresh packed chicken meat were not contaminated. Among the 32 isolates, 40.60%, 34.40%, 21.90%, and 15.60% were resistant to tetracycline, ciprofloxacin, ampicillin, and streptomycin, respectively. Moreover, a high number of multi-antibiotic resistant *Campylobacter* spp. was determined. Since foods of animal origin are the most sources of Campylobacter infection, the presence of resistant strains to antibiotics is a potential risk to public health.

شناسایی *کمپیلوباکتر کولای و کمپیلو باکتر ژژونی* مقاوم به چند آنتی بیوتیک در گوشت گوساله، گوسفند، جوجه و گاومیش در اهواز، ایران

چکیده

کمپیلو باکتر ژژونی و کمپیلوباکتر کولای، عوامل اصلی بیماری های معدی روده ای در انسان حتی در کشورهای صنعتی می‌باشند که بهداشت عمومی را تحت تأثیر قرار می دهند. هدف مطالعه حاضر، بررسی میزان وقوع و مقاومت آنتی بیوتیکی *کمپیلو باکتر ژژونی و کمپیلوباکتر کولای* در گوشت جوجه ، گوساله، گوسفند و گاومیش آبی کشتار شده در شهر اهواز، ایران بود. در مجموع ۳۸۰ نمونه شامل ۱۵۰ نمونه گوشت جوجه از کشتارگاه های صنعتی، ۵۰ نمونه گوشت جوجه از کشتارگاه های سنتی، ۳۰ نمونه گوشت جوجه بسته بندی شده تازه از بازارهای محلی و گوشت گوساله، گوسفند و گاومیش آبی (از هر کدام ۵۰ نمونه) از کشتارگاه های صنعتی در اهواز، جمع آوری شدند و از نظر حضور گونه های *کمپیلوباکتر* مورد بررسی قرار گرفتند. روند آزمایش شامل یک مرحله غنی سازی در محیط مایع غنی کننده پرستون و متعاقب آن کشت در محیط کشت آگار خون دار حاوی مکمل به مدت ۲۴ ساعت در شرایط میکروایروفیلیک در دمای ۴۲ درجه سانتیگراد بود. کلنی های مشکوک با روش واکنش زنجیره ای پلیمراز مورد بررسی قرار گرفتند و جدایه های تایید شده به روش انتشار دیسکی Kirby-Bauer از نظر حساسیت به آنتی بیوتیک های مختلف آزمایش شدند. در مجموع، ۳۲ نمونه (۸/۴۰ درصد) به گونه های *کمپیلوباکتر* آلوده بودند. گوشت گوسفند آلوده ترین گوشت (۲۴/۰۰ درصد) بود، در حالی که درگوشت جوجه بسته بندی شده تازه آلودگی مشاهده نگردید. از بین ۳۲ جدایه ۴۰/۶۰ ، ۳۴/۴۰ ، ۲۱/۹۰ و ۱۵/۶۰ درصد به ترتیب به تتراسایکلین، سیپروفلوکساسین، آمپی سیلین و استرپتومایسین مقاومت نشان دادند. همچنین، تعداد زیادی گونه *کمپیلوباکتر* مقاوم به چند آنتی بیوتیک شناسایی گردید. از آن جایی که مواد غذایی با منشا دامی بیشترین منابع آلودگی کمپیلوباکتر می باشند، حضور سویه های مقاوم به آنتی بیوتیک ها یک خطر بالقوه برای بهداشت عمومی محسوب می گردد.

واژه های کلیدی: جوجه،*کمپیلوباکتر*،گاومیش آبی، گوسفند، گوشت گوساله

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Introduction

Thermophilic Campylobacters such as *Campylobacter jejuni* and *Campylobacter coli* are one of the main causes of gastrointestinal diseases in humans even in industrialized countries affecting public health.[1, 2] The most predominant clinical symptoms of campylobacteriosis in humans are mild to severe bloody diarrhea, abdominal pain and sometimes fever. Most of patients recover after a week, but some cases may require treatment with antibiotics. Arthritis, meningitis, myocarditis, urinary tract infection, Guillain-Barre syndrome, and Miller Fisher syndrome are other complications in the acute phase of the disease.[3,4] The low infective dose of Campylobacter cells means that even very small numbers of *Campylobacter* spp. in food samples can be a potential hazard to human health.[5] Antibiotic therapy in animals and humans for various purposes has led to the increased bacterial resistance to many antibiotics. Antimicrobial-resistant strains could be transmitted to humans by the consumption of contaminated food and nowadays it has been a significant public health concern.[6] Although poultry have been introduced as the main source of campylobacteriosis in humans, there is evidence suggesting that other animals such as cattle and sheep frequently carry *C. jejuni* and *C. coli* in their intestine.[7-9] Meat may be contaminated by feces during the slaughtering process and then, consumption of the contaminated meat may pose a risk of Campylobacter transmission.[10,11] Monitoring animal meats for detecting antibiotics resistant foodborne pathogens is necessary.

Isolation of *Campylobacter* spp. from meat samples in various provinces such as Khorasan in north,[12,13] Fars in south,[14] Tehran in middle-north[15,16] and Kerman in central[17] of Iran has been reported during the last decade. There is a little information available about Campylobacter contamination of raw meats in Ahvaz located in south-west of Iran. The aim of the current study was to investigate the presence and antimicrobial susceptibility of *C. jejuni* and *C. coli* in chicken meat, beef, mutton, and water buffalo meat slaughtered in Ahvaz, Iran.

Materials and Methods

Sampling. In this cross-sectional survey during a six month period from January to July 2016, a total of 380 samples including chicken meat from industrial abattoirs (n = 150), chicken meat from traditional abattoirs (n = 50), fresh packed chicken meat from local markets (n = 30) and beef, mutton and water buffalo meat from industrial abattoirs (50 samples for each meat) were collected. In abattoir samples were taken after washing the carcasses and before transferring to the retailers. For Sampling, 10 g meat was taken from the surface section of flank and chuck and placed in a sterile falcon tube containing 30 mL buffered peptone water. Falcon tubes were well shaken and transported to the laboratory in a cooler with an ice pack in less than 2 hr.[18,19]

Isolation and Identification of *Campylobacter* spp. In the laboratory, tubes were centrifuged at 4000 rpm for 5 min. The supernatant was discarded and the pellet was dissolved in 30 mL Preston enrichment broth base (Himedia, Mumbai, India) supplemented with 5% defibrinated horse blood and an antibiotic vial (FD042; Himedia). Tubes were incubated under microaerophilic conditions at a temperature of 42.00 ± 0.50 ˚C for 24 hr. Then, 0.10 mL of the cultures were streaked onto individual selective agar plates (blood agar base supplemented with FD 006; Himedia) and incubated for 48 hr at 42 ˚C under the same conditions. Suspected colonies (flat, non-hemolytic, gray and circular) were subjected to gram staining, catalase, oxidase, nitrate reduction, and nalidixic acid resistance tests. Positive colonies were tested by polymerase chain reaction (PCR) assay for final confirmation.[20]

Polymerase chain reaction procedures. Presumptive colonies in two steps were subjected to the PCR assay. The first step was to confirm *Campylobacter* genus and the second one was to identify *C. jejuni* and *C. coli* species. Template DNA was obtained by boiling method[21] from a pure culture of the suspected isolates. Briefly, the bacterial culture was centrifuged and the pellet was re-suspended in 1 mL of deionized water. The sample was boiled at 100 ˚C for 10 min and the suspension was centrifuged at 14000 rpm for 10 min. The supernatant was used as a PCR template. According to Denis *et al.*,[22] three genes were selected for the identification of *Campylobacter* genus, *C. jejuni* and *C. coli* (Table 1).

Each PCR tube contained 25 µL of reaction mixture consisting of 20.00 µL master mix (CinnaGen, Tehran, Iran) and 5.00 µL of template DNA. The cycling conditions used in the thermal cycler (Bioer, Hercules, China) were as follow: the first denaturation at 95 ˚C (10 min, 1 cycle), denaturation at 95 ˚C for 30 sec, annealing at 59 ˚C for 90 sec and extension at 72 ˚C for 1 min. After 35 cycles, a final cycle comprised a 10 min extension step at 72 ˚C. The amplified PCR products were detected by agarose gel (1.20%) electrophoresis (Paya Pajoohesh Pars, Tehran, Iran), stained with 1.00% solution of ethidium bromide and visualized under ultraviolet light illumination (Kiagen, Tehran, Iran). Standard and confirmed strains of *C. coli* and *C. jejuni* (kindly provided by the University of Shiraz) were used as positive controls and deionized water was used as a negative control.

Antibiotic susceptibility testing. According to the method of Clinical and Laboratory Standards Institute,[23] antibiotic susceptibility tests were carried out using the Kirby-Bauer disk diffusion method. The tested antibiotic (Padtan Teb, Tehran, Iran) were ampicillin (10 mg), erythromycin (15 mg), ciprofloxacin (15 mg), tetracycline (15 mg), gentamicin (10 mg), chloramphenicol (30 mg),

Table 1. List of target genes, the sequence of primers and product size (bp).

Specificity	Gene	Size (bp)	Primer sequence
***Campylobacter* genus**	16S rRNA	857	Forward: ATC TAA TGG CTT AAC CAT TAA AC Reverse: GGA CGG TAA CTA GTT TAG TAT T
C. jejuni	*mapA*	589	Forward: CTA TTT TAT TTT TGA GTG CTT GTG Reverse: GCT TTA TTT GCC ATT TGT TTT ATT A
C. coli	*ceuE*	462	Forward: AAT TGA AAA TTG CTC CAA CTA TG Reverse: TGA TTT TAT TAT TTG TAG CAG CG

streptomycin (30 mg) and enrofloxacin (10 mg). A swab was taken from each bacterial suspension (almost equal to 1×10^8 CFU mL^{-1} or 0.50 McFarland standards) and stroked thoroughly on Mueller-Hinton agar (Mast Diagnostics, Merseyside, UK) with 5.00% defibrinated sheep blood agar,[24] and then antibiotic discs (Padtan Teb, Tehran, Iran) were placed on the agar. After incubation under a microaerophilic condition at 42 ˚C for 48 hr,[25] the diameter of the inhibition zone was measured for each antibiotic. Then, the isolates were classified as resistant, intermediate (reduced susceptibility) or sensitive.

Results

Among 380 meat samples, nine chicken meat (2.40%), seven beef (1.80%), four buffalo meat (1.10%) and 12 mutton (3.10%) samples were found to be contaminated with *Campylobacter* spp. The predominant species in samples were *C. jejuni* (81.30%) followed by *C. coli* (18.70%), (Fig. 1).

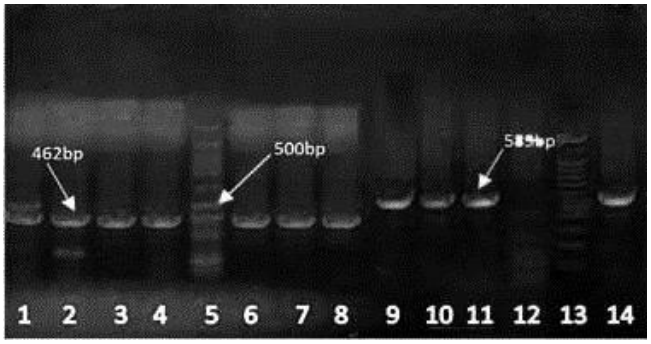

Fig.1. Polymerase chain reaction results for *C. jejuni* and *C. coli* detection on gel electrophoresis. Lane 1: Positive control 462 bp (*C. coli*); Lanes 2-4: *C. coli* isolates; Lane 5: Ladder 100 bp plus; Lanes 6-8: *C. coli* isolates; Lanes 9-11: *C. jejuni* isolates; Lane 12: Negative control; Lane 13: Ladder 100 bp plus; Lane14: Positive control 589 bp (*C. jejuni*).

Table 2 shows the prevalence of *Campylobacter* spp. isolated from chicken meat, beef, mutton and buffalo meat in Ahvaz, Iran. As can be seen in Table 2, the highest prevalence (24.00%) of *Campylobacter* spp. was found in mutton. Chicken meat obtained from traditional abattoirs and beef had the second and third prevalence values among samples, respectively. Interestingly, the fresh packed chicken meat showed no contamination to *Campylobacter* spp.

Antibiotic susceptibility tests were carried out on all *Campylobacter* spp. isolated in this study. The susceptibility, intermediate and resistance profiles of the isolates to eight tested antibiotics are shown in Table 3.

Overall, 40.60% were resistant to tetracycline, 34.40% were resistant to ciprofloxacin and 21.90% were resistant to ampicillin. Also, 15.60% of isolates were resistant to streptomycin. Surprisingly, no resistance to gentamicin was observed in the isolates.

Discussion

Campylobacter spp. is mainly transmitted to humans through contaminated foods of animal origin.[26] Contamination of food to thermophilic *Campylobacter* spp. could occur during production, processing, distribution, and preparation in the food supply chain.[26,27] Consumption of undercooked meat products contaminated with these pathogens has been identified as a risk factor for human campylobacteriosis.

In this study, 380 raw samples of chicken meat, beef, mutton and buffalo meat slaughtered in industrial and traditional abattoirs were examined. Overall, 8.40% of all the meat samples were contaminated with *C. coli* and *C. jejuni*. The results of this study showed that mutton (24.00%) and chicken carcasses slaughtered in traditional abattoirs (16.00%) are important sources for Campylobacter infections in Ahvaz, Iran. The prevalence of

Table 2. Prevalence of *Campylobacter* spp. isolated from chicken meat, beef, mutton and buffalo meat in Ahvaz, Iran.

Meat sample	Number of samples	*Campylobacter* spp. Positive (%)	*C. jejuni* (%)	*C. coli* (%)
Chicken meat from industrial abattoirs	150	1(0.60)	0(0)	1(100)
Chicken meat from traditional abattoirs	50	8(16.00)	3(37.50)	5(62.50)
Fresh packed chicken meat	30	0 (0)	0 (0)	0 (0)
Beef	50	7 (14.00)	7(100)	0 (0)
Mutton	50	12 (24.00)	12(100)	0 (0)
Water buffalo meat	50	4 (8.00)	4(100)	0 (0)
Total	380	32 (8.40)	26(81.30)	6(18.70)

Table 3. The resistance profile of *Campylobacter* spp. isolated from various meats.

Antibiotic	Chicken meat			Beef			Mutton			Buffalo meat			Total number of resistant isolates (%)
profile	S	I	R	S	I	R	S	I	R	S	I	R	
Ampicillin	6	1	2	5	-	2	10	-	2	3	-	1	7 (21.90)
Streptomycin	2	2	5	6	1	-	12	-	-	4	-	-	5 (15.60)
Gentamicin	9	-	-	7	-	-	12	-	-	4	-	-	0 (0)
Erythromycin	9	-	-	7	-	-	9	1	2	4	-	-	2 (6.20)
Ciprofloxacin	8	-	1	3	-	4	6	2	4	2	-	2	11 (34.40)
Tetracycline	5	-	4	4	-	3	7	-	5	3	-	1	13 (40.60)
Chloramphenicol	7	1	1	7	-	-	10	-	2	4	-	-	3 (9.40)
Enrofloxacin	8	1	-	6	-	1	9	2	1	4	-	-	2 (6.20)

S: Susceptible; I: Intermediate resistant; R: Resistant.

Campylobacter spp. in the beef, buffalo meat and chicken carcasses slaughtered in industrial abattoirs as other sources were 14.00%, 8.00%, and 0.60%, respectively.

To the best of our knowledge, there are several studies regarding isolation of *Campylobacter* spp. from various meat samples in Iran and in the world, but few reports could be found on the occurrence of *Campylobacter* spp. in the water buffalo meat. According to Andrzejewska *et al*. 41.60% of the poultry meats available in retail stores in the northern part of Poland were contaminated with *Campylobacter* spp.[28] In another study in this country, thermophilic *Campylobacter* species were detected in poultry (49.30%), pork (10.60%) and beef (10.10%).[29] In another study in northwestern Greece, *Campylobacter* spp. were isolated from 91 (29.40%) of the free-range chicken meat samples and from 106 (28.70%) of the conventional farming chicken meat samples,[30] while only one sample (0.60%) was contaminated with *C. jejuni* in 175 bovine ground meat samples in Finland.[31] It could be concluded that raw retail meats are potential vehicles for transmitting campylobacteriosis, however, the prevalence of these pathogens is markedly different in various meats.

In Iran, *Campylobacter* spp. were isolated from 110 (44.00%) of chicken meat samples and from 11 (5.50%) of beef samples,[16] which are much higher and lower than our results, respectively. The contamination rate of mutton samples observed in our study (24.00%) was also much higher than the results reported by Rahimi *et al.* (12.00%) in central of Iran.[25]

Comparison of data also indicated that the contamination rate in buffalo meat (8.00%) in our work was much lower than the previous data (21.40%) in Iran.[32] The difference in data suggests that time, season, place of sampling methods, and laboratory techniques used in studies may affect the results of prevalence rate. It seems that humidity and precipitation were not important predictors for Campylobacter prevalence; meanwhile a strong relationship between temperature and Campylobacter infections has been demonstrated.[33]

In our study, *C. jejuni* was the most prevalent *Campylobacter* spp. recovered from the samples which is in agreement with reports of Dabiri *et al*. in Iran,[16] Guyard-Nicodème *et al.* in France[34] and Han *et al.* in China.[35]

It is noticeable that slaughtering, evisceration, and skinning of large animals in Ahvaz abattoir are manual and cross-contamination during these procedures could happen. Comparison of the poultry slaughtered in industrial abattoirs and those that have been slaughtered traditionally in our study showed that slaughterhouse sanitation process could be effective in the elimination or reduction of Campylobacter in poultry meat.

The results of antimicrobial susceptibility testing in the present study showed that 41.70% (5/12) and 33.30% (4/12) of strains isolated from mutton were resistant to tetracycline and ciprofloxacin, respectively. Surprisingly, 40.60% and 34.40% of all Campylobacter isolates were resistant to these antibiotics. All isolates were sensitive to gentamicin which is in agreement with previous reports.[36,37] On the contrary of results reported by Van Looveren *et al*. and Ge *et al.* a low resistance to erythromycin and enrofloxacin (6.20%) was displayed in our study.[38,39]

It is noticeable that we found a high number of multi-antibiotic-resistant *Campylobacter* spp. in different meats, mostly in mutton which has the highest interest for consumption in Iran. The existence of such strains in meat has important public health and health promotion policy implications. It is recommended that in patients with campylobacteriosis, especially in immunosuppressive cases or for those cases with severe or prolonged symptoms, an antibiotic susceptibility testing of Campylobacter can be performed and appropriate antibiotics be prescribed.

The presence of resistant strains to antibiotics in meat and other foods should be taken seriously and hygienic measures are necessary to be taken in this regard. Antibiotics prescription in livestock and poultry under the supervision of a veterinarian, considering mandatory antibiotic withdrawal times before slaughtering, application of a fully sanitized procedure during the slaughtering, permanent microbiological monitoring in abattoirs and carcasses, inhibiting the activity of traditional slaughterhouses, sanitation education of the public in abattoirs, retailers, restaurants and home environments and fully cooking of raw meat can be useful in reducing Campylobacter infection risk.

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Conflict of interest

The authors declare that there is no conflict of interest.

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