JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH, 2020
VOL 11, NO. 1
10.5455/jcmr.2020.11.01.34

RESEARCH ARTICLE

Open Access

Prevalence, Virulence and Anti-Microbial Resistance in *Campylobacter spp*. from Routine Slaughtered Ruminants, as a Concern of Public Health (Case: Chaharmahal and Bakhtiari Province, Iran)

Amir Shafiei [1], Ebrahim Rahimi[1*], Amir Shakerian[2]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[2]Nutrition and Organic Products Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
*Corresponding author: Ebrahim Rahimi, Department of Food Hygiene, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran, E-mail: ebrahimrahimi55@yahoo.com

ABSTRACT

In present study, the prevalence and infection rate of *Campylobacter spp*. was assessed in 1800 samples; 360 ruminant in the Chaharmahal and Bakhtiari province, over a 12-month period between September 2018 and September 2019. Samples were more contaminated with *Campylobacter jejuni* (3.2%), than with *Campylobacter coli* (2.5%). Of 114 isolates of Campylobacter shown resistance to one or more of the twelve antimicrobials compared with 64 (79.2%) of 114 isolates of *C. jejuni* .The frequency of resistance between isolated ones was statistically significant across divisions. Overall, the resistance was in greater rate to Tetracycline (65.7%) and Ciprofloxacin (50.0%) and lowest to Imipenem (2.6%) and the differences were significant ($P < 0.05$).The presence of the *cadF , flaA, cdtB, cdtA, cdtC* among 64 *C. jejuni* and 45 *C. coli* isolates was identified by PCR method. The high prevalence of five virulence genes indicates that these putative pathogenics determinants are widespread among *Campylobacter* which isolates from ruminant such as cows, goats and sheep.

ARTICLE HISTORY

Received May 21, 2020

Accepted June 10, 2020

Published August 01, 2020

KEYWORDS

Campylobacter jejuni, PCR, *Virulence gene, Antimicrobial resistance*.

*Contact: Ebrahim Rahimi *Department of Food Hygiene, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran - Tel: +9133278377* ebrahimrahimi55@yahoo.com

INTRODUCTION

Food related diseases are one of the most important problems in societies and may have considerable economic hazards (11 ،9 ،15 ،19 ، 23). Campylobacters are bar formed, non-sporogenic Gram-negative living beings having a place with the Enterobacteriaceae family and are one of the most pervasive harming factors normal among people and creatures having hurtful connections among people and creatures with various species and hosts (13 ،16 ،21 ،30 ،35). In the *Campylobacteriaceae* family, two important species, *jejuni* and *coli* are responsible for most cases of C*ampylobacter* infections in human communities worldwide (7 ،18 ،24 ،29). Global statistics indicate that 2 to 35 percent of bacterial diarrhea is caused by this pathogen in different communities, which this amount is multiple times the amount of infection in human societies to *Salmonella,* indicating the importance of this pathogen in human health. with the increasing trend of urban life and industrialization of societies and increasing public awareness of proper nutrition, people's amount of consumption of meat of livestock and slaughter poultry as a protein source is increasing that is supplied often from meat of livestock such as beef, lamb and poultry (17 ،20 ،27 ،33).

Albeit, much consideration has been centered around poultry meat, red meat additionally remains the most well-known reason for food borne general flare-ups of irresistible intestinal sickness (3, 8). There is restricted data on the pervasiveness of Campylobacter in crude meat in Iran. Infection brought about by Campylobacter for the most part shows at loose bowels, fever and serious stomach torment. Albeit, most human cases are inconsistent and flare-ups are moderately uncommon (26), increasingly genuine results of campylobacteriosis incorporate the immune system intervened demyelinatiog neuropathies Guillain-Barre and Miller Fisher conditions (25). Another issue of concern with respect to Campylobacter is the expansion in antimicrobial opposition showing up in different locales around the globe (28). Disease to these Campylobacters may prompt problematic results of antimicrobial treatment (32) or treatment disappointment (10). Antimicrobial obstruction in both human and animal Campylobacter detaches has gotten progressively basic in Thailand (12). A prior examination in Thailand discovered high extents of Campylobacter impervious to an assortment of antimicrobial operators, including fluoroquinolones (nalidixic corrosive and ciprofloxacin) (42)

In spite of the fact that destructiveness instruments in *Campylobacter. spp* are not totally known, various putative destructiveness and poison qualities have been distinguished so far utilizing the sub-atomic science techniques (7). Bacterial flagellum is the most noteworthy harmfulness factors, which are identified with motility, grip, and attack. FlagellinA (flaA) is liable for chemo taxis and also adherence. Campylobacter attach to fibronectin (cadF) is another factor which is at risk for adherence. Destructiveness characteristics associated with Campylobacter rudeness are the assault related marker (iam) characteristics, including Phospholipase A (pladA, etc (44-51). A couple of diseases have in like manner been recognized in Campylobacter, among which cyto-deadly distending tainting (CDT) has been developed to be destructive for have enterocytes (7-8).

MATERIALS AND METHODS

Samples

From September 2018 to September 2019, a total of 1800 samples from slaughtered ruminants caw (n = 600), sheep (n = 600), and goat (n = 600) were obtained from randomly-selected four slaughterhouses in Saman, Lordegan and Joneghan, and Farrokhshahr, in Chaharmahal and Bakhtiari province, Iran. The samples included meat, liver, kidney, heart and contents of rectum. All examples were set in independent clean plastic sacks to forestall spilling and cross defilement and were promptly moved to the research facility in a cooler with ice packs.

Microbiological assays

The examples were prepared promptly upon landing in the lab by utilizing aseptic procedures Each example (10 g) was homogenized and moved to 90 mL Campylobacter Enriched Broth (Preston advancement stock base, Himedia, Mumbai, India, M899) was enhanced with the chose Campylobacter supplement (Himedia, Mombia, India, FD042) and 25 ml of defibrinated sheep blood were included per each 475 ml of medium. After 24 h hatching, 0.1 ml of it on the particular media of Campylobacter (Himedia, Mumbai, India, M994) was improved with anti-infection supplements (Himedia, Mumbai, India, FD006) and5% (v/v) defibrinated sheep blood and brooded at 42°C for 48 h under a similar condition. One possible Campylobacter settlement from each specific agar plate was subculture and tried by standard small scale natural and biochemical systems. Single-developing provinces were concentrated to affirm and isolate Campylobacter species as far as warm recoloring, catalase creation, oxidase, hydrolysis of Hippurate and protection from cephalothin (9). Settlements suspected to Campylobacter were browsed every particular agar plate and presented to recognizing verification as demonstrated by the standard

microbiological and biochemical tests including microscopic morphology, Gram recoloring, production of catalase, oxidase, maturing of glucose, nitrate decline, and hippurate hydrolysis (7).

Extraction of DNA and PCR condition

The DNA was removed for PCR by the traditional bubbling technique. Rapidly, one area of each unadulterated culture plate was suspended in 200 µL refined water and warmed at 95°C for 10 min in thermocycler, after which the suspension was centrifuged at 10000 rpm for 10 min, by then the supernatants were taken care of at −20°C and used as format DNA (10-11). The character of the disconnects was asserted by Polymerase chain reaction (PCR) using starters express for cadF, and characteristics which unequivocally perceive Campylobacter spp. Tallying C. coli and C. jejuni species, independently (Table 1) (12). The PCR reaction mix was contained 3 mL of each expelled DNA, 2.5 µL of 10x PCR support, 0.3 mL of 10mMdNTP mix, 25 pmol of all of fundamentals, and 0.6µL MgCl2 (50 mM), 1U of Taq DNA polymerase and deionized water to a last volume of 25 µL. The escalation reaction was acted in a thermocycler structure (Mastercycler incline, Eppendrof, Germany). The going with PCR conditions were used: starting denaturation at 95°C for 5 min; 30 cycles with denaturation at 95°C for 45s; hardening at 49°C for iam, 43°C for cadF, 45°C for pldA and flaA and cdtA for 1 min; and increase at 72°C for 1 min; with the last extension at 72°C for 5 min. Finally, the isolates were perused for the proximity of five pathogenic characteristics. Preparation progressions were gotten from recently organized primers (Table 1) (Table 2) (8, 12-15). The C. jejuni ATCC 29428 and C. coli ATCC 43478 strains were used as controls in each PCR measure (9). DNA of the affirmed provinces dependent on culture utilizing the DNA extraction pack (Cinna Gen, Iran) was removed by the unit producer's guidelines. The PCR test technique in this examination was performed by the strategy portrayed by Denis et al. (1999). To lead the PCR response, the last response volume was viewed as 25 microliters, including 20 ng of format DNA, 2 mM MgCl2, 25 picomol of every groundwork, one Taq polymerase chemical unit, and 200 µM dNTP blend. Table 1 shows the size of the PCR item for each example. To affirm the nearness of intensified piece, 20 µl of the PCR item was electrophoresed on 1.5% agarose gel containing ethidium bromide within the sight of 100 bp DNA marker at a consistent voltage of 80 V.

Table 1: PCR primers used to detect *Campylobacter* genus and *Campylobacter* species: *jejuni* and *coli*

gene	primer sequence	product size	reference
16SrRNA	MD16S1 upper primer 5′ AT C TAA T GG CTT AAC CAT TAA AC 3′ MD16S1 lower primer 5′ GGA CG G TAA CTA GTT TAG TAT T 3′	857 bp for Campylobacter genus	12
mapA	MDmapA1 upper primer 5′ CTA TTT TAT T TT TGA GTG CTT GTG 3′ MDmapA2 lower primer 5′ GCT TTA T TT GCC ATT TGT TTT ATT A 3′	589 bp for C. jejuni	19
ceuE	COL3 upper primer 5′ AAT TGA A AA TTG CTC CAA CTA TG 3′ MDCOL2 lower primer 5′ TGA TT T TAT TAT TTG TAG CAG CG 3′	462 bp for C. coli	7

Table 2: Primers used to trace *Campylobacter* virulence genes and *Campylobacter* species: *jejuni* and *coli*

Primers	Sequences (amplicon sizes)	PCR conditions
cadF gene	F2B: 5'-TG GAGGGTAATTTAGATATG-3' RIB: 5'- CT AATACCTAAAGTTGAAAC-3' (Amplicon: 400bp)	94°C 1 min (30cycles) 45°C 1 min 72°C 3 min
ceuE gene (For *C.jujeni*)	JeJt: 5'-CC TGCTCGGTGAAAGTTTTG-3' JeJ2: 5'- GA TCTTTTTGTTTTGTGCTGC-3 (Amplicon: 794 bp)	93°C 3 min 95°C 30 s
ceuE gene (For *C. coli*)	COL1: 5ATGAAAAAATATTTAGTTTTTGGA3' COL2: 5'-ATTTTATTATTTGTAGC.AGCG-3' (Amplicon: 894 bp)	57°C 30 s (30 cycles) 72°C 1 min

flaA gene	fla A-F: 5'-GGAAATTGGATTTGGGGCTATACT-3' fla A-R: 5'- CTGTAGTAATCTTAAAACATTTTG-3' (Amplicon: 1728 bp)	94°C 1 min 45°C 1 min (30 cycles) 72°C 3 *min*
Cdt A gene	GNW: 5'-GGAAATTGGATTTGGGGCTATACT-3' IVH: 5'- ATCACAAGGATAATGGACAAT-3' (Amphcon: 165 bp)	
cdtB gene	VAT2l: 5' GTTAAAATCCCCTGCTATCAACCA 3' WMI-R 5' GTTGGCACTTGGAATTTGCAAGGC3' (Amplicon: 555bp)	
Cdt genes cluster	GNW and LPF-X) (Amphcon: 1215 bp)	
Cdt genes	LYA-f: 5'-CTTTATGCATGTTCTTCTAAATTT-3' MII-R: 5'-GTTAAAGGTGGGGTTATAATCATT-3' (Amplicon: 2212 bp)	

According to the protocol of Clinical and Laboratory Standards Institute, Antimicrobial susceptibility test was performed using the disk diffusion method on Muller Hinton medium (HiMedia, Laboratories, Mumbai, India) enriched with 5% sheep defibrinated blood, according to the method provided by CLSI (Clinical and Laboratory Standards Institute, 2006). The antibiotic discs used in this study were manufactured by Indian HiMedia companies (HiMedia, Laboratories, Mumbai-India). The type and concentration of each antibiotic used are: Nalidixic Acid (30 ug), Ciprofloxacin (5 ug), Erythromycin (15 ug), Tetracycline (15 ug), Streptomycin (30 ug), Ampicillin (10 ug), Amoxicillin (30 ug), Gentamicin (10 ug), and Chloramphenicol (30 ug). After culturing and disking at 42 °C under microaerophilic conditions for 48 hours, the plates were incubated. After incubation, non-growth areas around antibiotic discs were measured by a KT model caliper made in China.

Statistical analysis were conducted using SPSS software 16.0 (SPSS Inc-Chicago, IL.), chi-square test and fisher's exact two tailed test analysis were performed; $P < 0.05$.

RESULTS

Out of 1800 examples from 360 carcasses114 (6.3%) secludes were recognized as Campylobacter. spp dependent on biochemical and microbiological tests. Of these segregates, 69 (60.52 %) species were recognized as C. jejuni and 45 (39.48%) as C. coli. Campylobacter was separated from an essentially bigger number of sheep's corpses 72 (63.1 %) contrast with goat's bodies 27 (23.6 %) and dairy animals' bodies 15 (13.1%) ($P < 0.05$). The results have indicated the presence of *Campylobacter.spp* in 64(3.5%) of the samples. Frequency of *C. jejuni* in the examined samples was2.5%. *C. coli* were found in 4.0% of the analyzed samples. The test uncovered that *C. jejuni* confines were essentially more much of the time identified than *C. coli* disconnects in a wide range of the inspected samples. ($p < 0.5$). The samples from Contents of rectum had the highest prevalence of Campylobacter (42.1% in 1years). The proportion of Campylobacter-positive samples varied among various sample types, from 0% (goat kidney, cattle kidney and cattle heart) to 20% sheep contents of rectum. *Campylobacter .spp* was recognized in 60.8 % of cadavers of sheep as a rule it was distinguished as *C. jejuni*. Through the span of our examination, the most reduced pervasiveness of the inspected microorganisms was seen in 4.2% steers corpses, in goat remains (21.7%).

Table 3: Distribution/prevalance of *campylobacter* isolates across various carcass samples

Sample source	Number of samples collected	Number of positive samples(%)	*Campylobacter coli*(%)	*Campylobacter jejuni* (%).
Cattle meat	120	3(2.5)	3(6.6)	0 (0)
Cattle liver	120	3(2.5)	3(6.6)	0 (0)
Cattle kidney	120	0 (0)	0 0)	0 (0)

Cattle heart	120	0 (0)	0 (0)	0 (0)
Cattle Contents of rectum	120	9 (7.5)	6 (13.3)	3 (4.3)
Subtotal(A)	600	15(2.5)	12(26.6)	3 (34.4)
Sheep meat	120	15(12.5)	6 (13.3)	9 (13.4)
Sheep liver	120	21(17.5)	6 (13.3)	15(21.7)
Sheep kidney	120	9 (7.5)	3 (6.6)	6 (8.6)
Sheep heart	120	3(2.5)	3(6.6)	0(0)
Sheep Contents of rectum	120	24 (20)	12(26.6)	12(17.3)
Subtotal(B)	600	72 (12)	30 (66.6)	42(60.8)
Goat meat	120	3 (2.5)	0 (0)	3(4.3)
Goat liver	120	18(15)	9(20)	9(13)
Goat kidney	120	0 (0)	0(0)	0 (0)
Goat heart	120	3 (2.5)	3 (6.6)	0 (0)
Goat Contents of rectum	120	15(12.5)	6(13.3)	9(13)
Subtotal(C)	600	27(4.5)	12(26.6)	15 (21.7)
Total	1800	114(6.3)	45(100)	69(100)

The PCR for recognition of cadF and flaA positive for cadF, and flaA qualities (Table. 9, Table 10) harmfulness qualities demonstrated that100% of the secludes were certain for cadF and flaA. All Campylobacter spp. secludes from butchered creatures had cadF quality, liable for adherence.

Table 4: prevalence of virulent gens in *Campylobacter* isolated recovered from various sources

Source	**Number of isolates**	**Virulence genes detected in campylobacter spp.** ***cadF***	***flaA***	***Cdt A***	***Cdt*** B	***Cdt C***
Cattle meat	3	3(100)	3(100)	2(66.6)	1(33.3)	2(66.6)
Cattle liver	3	3(100)	3(100)	2(66.6)	2(66.6)	1(33.3)
Cattle kidney	0	0(100)	0(0)	0(0)	0(0)	0(0)
Cattle heart	0	0(0)	0(0)	0(0)	0(0)	0(0)
Cattle Contents of rectum	9	9(100)	9(100)	6(66.6)	8(88.8)	7(77.7)
Sheep meat	15	15(100)	15(100)	11(73.3)	9(60)	6 (40)
Sheep liver	21	21(100)	21(100)	18 (55.5)	12 (57.1)	10 (47.6)
Sheep kidney	9	9(100)	9(100)	8(88.8)	5 (88.8)	6 (66.6)
Sheep heart	3	3(100)	3(100)	2(66.6)	1 (33.3)	1 (33.3)
Sheep Contents of rectum	24	24(100)	24(100)	22 (91.6)	16(66.6)	14(58.3)
Goat meat	3	3(100)	3(100)	2(66.6)	1 (33.3)	1 (33.3)
Goat liver	18	18(100)	18(100)	16(88.8)	12(66.6)	10(55.5)
Goat kidney	0	0(0)	0(0)	0(0)	0(0)	0(0)
Goat heart	3	3(100)	3(100)	2(66.6)	1 (33.3)	2 (66.6)
Goat Contents of rectum	15	15(100)	15(100)	12(80)	9(60)	11(73.3)
Total	114	114(100)	114(100)	97(85)	77(67.5)	91(79.8)

Despite species recognizing confirmation, all the separates were sure for cadF (Campylobacter connection to fibronectin) quality which urges adherence to fibronectin in the gastrointestinal epithelial cells of the animals (49). Moreover, the cadF quality furthermore accept a huge activity in the assault of the epithelial cells .This quality is mediated by a 37-kDa fibronectin-definitive out layer protein and is crucial for Campylobacter adherence to and colonization of the host cell surface. (57 ،73).

The current examination like an others reviews indicated a high predominance (100%) of the cadF quality, which shows that the lion's share confines beginning from the contemplated domesticated animals tests have the high danger of pathogenicity in Campylobacter .spp of the domesticated animals creation. (76،78).

The high power of cadF quality is a direct result of the way that this quality advances tiny living beings have cells collaboration and it has been depicted as a spared and sort unequivocal quality (47 ،50 ،60). The putative danger characteristics fuse cytolethal distending poison (CDT), similarly as cdtA, cdtB, and cdtC, poison characteristics encoding for Campylobacter cytotoxins. Cytotoxin made by Campylobacter. spp causes DNA wounds, chromatin crack, cytoplasm distension and cell

cycle catch in the G2/M change stage, inciting dynamic cell distension and in the end, cell passing (48). The damaging tendency of Campylobacter. spp is connected with the making of cytotoxins, where, in the current assessment all the investigated limits held the cytotoxicity characteristics cdtA, cdtB, and cdtC, the low inescapability of cdtA, cdtB, and cdtC characteristics in cows confines was viewed. While, in the examination that was driven a high regularity of these characteristics from separates was represented, the differentiations may be a direct result of genetic withdraws was represented, the qualifications may be a result of inherited segments, intermittent factors, types and number of tests, restriction techniques and transport conditions in the withdraws similarly found high inescapability of cdtA and cdtB from tests. Of course, in this audit found high regularity of cdtA, cdtB, and cdtC characteristics in goat liver withdraws. Regardless, found 60% in all the cdts characteristics in the ruminants isolates, this disclosures further avowed that al the three characteristics things are required for the toxic substance to be totally for all intents and purposes unique (12). This survey show high normality of the cytotoxicity (cdts) characteristics in sheep tests. Regardless, the high inescapability of danger factors found in the current assessment include the prerequisite for continued with general prosperity checking and observation of Campylobacter hurtfulness characteristics in different condition from animals and food, to help early acknowledgment of destructiveness characteristics especially in animal development and to evaluate the impact of strategies planned to diminish the prevalence of hurtfulness characteristics in creatures since it makes food pollution individuals. The utilization of one-prosperity approaches is basic to screen and diminish the impacts of prosperity threats across individuals, animals, cultivating and environmental interfaces.

Table 5: Distribution of virulent genes among of *campylobacter* isolates

Source	*Campylobacter spp*	Total number of isolates	Virulence genes detected in *campylobacter. spp*				
			cadF	*flaA*	*Cdt A*	*cdt B*	*Cdt C*
Cattle meat	***C.jejuni***	0	0	0	0	0	0
	C.coli	3	3	3	2	1	2
Cattle liver	***C.jejuni***	0	0	0	0	0	0
	C.coli	3	3	3	2	2	1
Cattle kidney	***C.jejuni***	0	0	0	0	0	0
	C.coli	0	0	0	0	0	0
Cattle heart	***C.jejuni***	0	0	0	0	0	0
	C.coli	0	0	0	0	0	0
Cattle Contents of rectum	***C.jejuni***	3	3	3	3	4	3
	C.coli	6	6	6	3	4	7
Sheep meat	***C.jejuni***	9	9	7	5	5	3
	C.coli	6	6	4	4	4	3
Sheep liver	***C.jejuni***	15	15	15	12	8	4
	C.coli	6	6	6	6	4	2
Sheep kidney	***C.jejuni***	6	6	6	5	3	4
	C.coli	3	3	3	3	2	2
Sheep heart	***C.jejuni***	0	0	0	0	0	0
	C.coli	3	3	3	2	1	1
Sheep Contents of rectum	***C.jejuni***	12	12	12	12	8	9
	C.coli	12	12	12	10	8	5
Goat meat	***C.jejuni***	3	3	3	2	1	1
	C.coli	0	0	0	0	0	1
Goat liver	***C.jejuni***	9	9	9	10	8	6
	C.coli	9	9	9	6	4	3
Goat kidney	***C.jejuni***	0	0	0	0	0	0
	C.coli	0	0	0	0	0	0
Goat heart	***C.jejuni***	0	0	0	0	0	0
	C.coli	3	3	3	2	1	2
Goat Contents of rectum	***C.jejuni***	9	9	9	8	7	8
	C.coli	6	6	6	4	2	3

C.jejuni= campylobacter jejuni C.coli= campylobacter coli

Antibiotic susceptibility-test against 12 antimicrobials was done for 114 isolates (69 *C. jejuni* and 45 *C. coli*) (Table 4). Seventy nine (28.9%) isolates were resistant to at Erythromycin. The greater rate of resistance (65.7 %) was seen against tetracycline. erythromycin (28.9%), meropenem (10.5%) imipenem (2.6%), amoxicillin (34.2%), ampicillin (47.3%), ciprofloxacin (50%) norfloxacin (18.4%), amikacin (15.7%), gentamicin (10.5%), cefazolin (39.4%) and streptomycin (18.4%). According to the *Campylobacter jejuni* the highest rate of resistance (82.6%) was seen against tetracycline. erythromycin (30.4%), meropenem (13.0%) imipenem (4.3%), amoxicillin (43.4%), ampicillin and ciprofloxacin (73.9%) norfloxacin (17.3%), amikacin (13.0%), gentamicin (39.1%), cefazolin (39.4%) and ctreptomycin (52.1%)

According to the *Campylobacter coli* the highest rate of resistance 40% was seen against tetracycline. The lowest rate of resistance (0%) was seen against imipenem, erythromycin (26.6%), meropenem and ampicillin (6.6%), amoxicillin, norfloxacin, amikacin and gentamicin (20%), ciprofloxacin and Streptomycin(13.3%)

Table 6: Number / Percentages of antimicrobial resistant Campylobacters Isolated From samples collected at the slaughterhouses

Type of antibiotic	Positive *Campylobacter*(n=114)		Positive *Campylobacter jejuni* (n=69)		positive *Campylobacter coli* (n=45)	
	Frequency	***Percentage***	***Frequency***	***Percentage***	***Frequency***	***Percentage***
Erythromycin	33	28.9	21	30.4	12	26.6
Meropenem	12	10.5	9	13.0	3	6.6
Imipenem	3	2.6	3	4.3	0	0
Amoxicillin	39	34.2	30	43.4	9	20
Ampicillin	54	47.3	51	73.9	3	6.6
Ciprofloxacin	57	50	51	73.9	6	13.3
Norfloxacin	21	18.4	12	17.3	9	20
Amikacin	18	15.7	9	13.0	9	20
Gentamicin	36	10.5	27	39.1	9	20
Tetracycline	75	65.7	57	82.6	18	40
Cefazolin	45	39.4	36	52.1	9	20
Streptomycin	21	18.4	15	21.7	6	13.3

Antimicrobial obstruction is the limit of a microorganism to oppose the development inhibitory or executing action of an antimicrobial past the ordinary helplessness of the particular bacterial species. Human campylobacteriosis for the most part frees from its own understanding without treatment. On the off chance that antimicrobial treatment is required, the most widely recognized medications utilized are macrolides, for example, erythromycin, and fluoroquinolones, for example, ciprofloxacin (6 ، 31، 36، 38). Expanding opposition of campylobacters to antimicrobials, particularly to fluoroquinolones, has been accounted for in segregates from the two creatures and ،Risk evaluation of Campylobacter spp. The improvement of protection from fluoroquinolones among campylobacters has happened simultaneously with the broad utilization of these antimicrobials in food creation creatures (52 ،58). Fluoroquinolone obstruction in campylobacters has constrained their convenience as a medication of decision in the treatment of human disease in numerous nations. Essentially, protection from macrolides is expanding in a few Campylobacter. spp detaches, especially in C. coli; nonetheless, erythromycin opposition in human detaches is still moderately low. Besides, gentamicin likewise stays powerful against campylobacters, in spite of the fact that it would typically be viewed as just for genuine Campylobacter. spp contaminations. Campylobacters are the most widely recognized zoonotic microbes segregated from solid dairy cattle (5،14). Cows are typically symptomless transporters of Campylobacter. spp (2 ، 21). The shedding of the living being can differ between singular creatures, which can be tireless or irregular shedders (22 ، 34). In this study decided the commonness of thermophilic Campylobacter spp. in cow rectal fecal examples from 5 slaughterhouses from 2018 to 2019. The complete commonness of Campylobacter.spp in tests was 56 %. C. jejuni, the most well-known species, was available in7.1 % of the examples among campylobacter. spp detached from food creation creatures the degree of the opposition of ciprofloxacin nalidixic corrosive and antibiotic medication are additionally commonly high. (4). All in all, the current examination features the occasional varieties in the predominance pace of Campylobacter spp a significant foodborne microbe having critical zoonotic significance

around the world. With a frequency pace of 22.72%, the most elevated predominance of C. jejuni was accounted for during rainseason followed by summer and winter

Table 7: Seasonal variation on prevalence of *campylobacter* in samples collected from slaughtered ruminants in Chaharmahal and Bakhtiari Province

Months of the Year	No of samples tested	No. of found positive	Percentage positive
Rainy season			
July2018	150	14	20%
August2018	150	16	22%
September2018	150	10	16%
October2018	150	8	10%
Subtotal(A)	600	48	17%
Winter			
November2018	150	6	6%
December2018	150	6	6%
January2019	150	6	6%
February2019	150	9	8%
Subtotal(B)	600	27	6.5%
Summer			
March2019	150	5	6%
April2019	150	9	12%
May2019	150	14	18%
June2019	150	11	16%
Subtotal(C)	600	39	12.5%
Total	1800	114	12%

C. jejuni is the most common cause of gastroenteritis or enterocolitis in man, especially in developed countries (45، 54، 66). Ruminants meat is a significant source of human gastroenteritis due to lack of care in handling raw products and inadequate cooking. Thus reduction of the risk to human health from Campylobacter contaminated sheep is a priority. An incidence rate of up to 60% in, cattle and goat, and up to 100% in chickens have been reported in various countries (70 ،77).

Pathogenic *Campylobacter. spp* was detected with relatively high frequency in India and Iran, which increases the risk of infections among the people living and working in farms (46 ، 59). Chicken, goat, sheep and cattle are major vehicle of *C. jejuni* and *C. coli* in developing countries (37، 43), however the authors of this study believed that climate and relative humidity affected the population of *campylobacters* in the environment. Therefore population of *campylobacters* in the environment is depended on the weather status of the countries. Existence of campylobacters in the intestinal tract of animals depended on their diet and intestinal tracts conditions. There is still a lot to be comprehended about the conduct and pathogenicity of these exceptionally significant microorganisms (40،56،67). From a food industry/sanitation point of view, it is imperative to all the more likely comprehend the conduct of C. jejuni and C. coli in the food creation condition, and how this influences their capacity to endure certain food creation forms.

Table 8: Seasonal variation on prevalence of *Campylobacter spp.* isolated from collected samples from slaughters of in Chaharmahal and Bakhtiari

Type of sample	season	N. sample	N. positive Campylobacter sample	N. positive Campylobacter Coli sample	N. positive Campylobacter Jejuni sample
Meat	**Cold**	180	6	3	3
	Hot	180	15	3	12
	Total	360	21	6	15
Liver	**Cold**	180	9	6	3
	Hot	180	21	3	18

	Total	360	30	9	21
Kidney	**Cold**	180	0	0	0
	Hot	180	9	3	6
	Total	360	9	3	6
Heart	**Cold**	180	3	3	0
	Hot	180	3	3	0
	Total	360	6	6	0
Contents	**Cold**	180	21	15	6
of rectum	**Hot**	180	27	6	21
	Total	360	48	24	24

End In this investigation, we showed that ruminant's meat can go about as significant wellsprings of human and ecological defilement by Campylobacter. spp In Chaharmahal and Bakhtiari region . Pollution of butchered ruminants demonstrates the need to apply great cleanliness rehearses in the butchering procedure and in meat dealing with. The absence of cleanliness in meat taking care of at the deal, cooking focuses, and butcher locales added to expanded cross-sullying through live creatures, meat taking care of, butchering, and cooking equipment(75،78). In spite of the fact that the viability of sub-atomic techniques, for example, PCR in the complete distinguishing proof of Campylobacter species, PCR has not regular been applied in food research centers in Iran. Subsequently, we can prescribe to general wellbeing authorities to incorporate this strategy as another option or a supplement to customary culture techniques

Examination in regards to the destructiveness markers of possibly pathogenic microscopic organisms, for example, Campylobacter strains in household creatures and in food with creature beginning is indispensable to shoppers' security. For this reason, we explored the conveyance of five destructiveness related qualities of Campylobacter strains disconnected from meat of butchered ruminants. The current examination demonstrated a high commonness rate for three out of five harmfulness qualities including cdtA, cadF in the entirety of the confines. Then again, all the disengages were certain for pladA and flaA qualities.

The nearness of safe strains to anti-toxins in meat and different nourishments ought to be paid attention to and clean measures are important to be taken in such manner. (41 ،53،63 ،65).

Anti-toxins remedy in animals of ruminants under the oversight of a veterinarian, considering obligatory anti-infection withdrawal times before butchering, utilization of a completely disinfected technique during the butchering, perpetual microbiological checking in corpses, repressing the action of conventional slaughterhouses, sanitation instruction of the open eateries and home situations and completely cooking of crude meat can be valuable in decreasing Campylobacter contamination hazard. (61 ،72،78).

It is recognizable that butchering, gutting, and cleaning of enormous creatures in some conventional slaughterhouses are manual and cross-tainting during these methodology could occur. Correlation of the ruminants butchered in mechanical abattoirs and those that have been butchered customarily in our examination demonstrated that slaughterhouse sanitation procedure could be successful in the disposal or decrease of Campylobacter in meat of sloughed ruminants (62 ،68،71).

All in all, this investigation has given data about the predominance of antimicrobial obstruction in Campylobacter from food animals at various stages in the chain from ranch to butcher in Chaharmahal and Bakhtiari Province. There were significant contrasts in the pervasiveness of safe Campylobacter among animals at the homestead for all operators tried, and between examining areas for most specialists tried. The expanded pervasiveness of safe disengages from meat tests gathered at advertise, contrasted with separates gathered from creatures at the slaughterhouse, proposes that defilement of nourishments of creature starting point after cadavers leave the slaughterhouse is a significant factor in the spread of safe microscopic organisms to the human evolved way of life. Following changes in antimicrobial vulnerability in Campylobacter from food creatures and food of creature roots was past the extent of this investigation; be that as it may, these findings show territories where future exploration can be focused to distinguish specific elements to decrease the pervasiveness of safe microscopic organisms entering the human food flexibly. (39 ،55،64).

The outcome indicated that a high extent of goat and sheep meat in Iran is debased with Campylobacter, especially with Campylobacter jejuni. The high pace of pollution in ruminant's meat alerts a huge general wellbeing concern. The vast majority of the disengages were safe; in this manner, there is a potential danger of human contamination with Campylobacter spp. by means of utilization of these items (69،74).

DISCUSSION

Despite the fact that nearness of polymorphisms in the groundworks toughening areas may not be precluded, while all C. jejuni introducing the cdt operon had the 3 segments, a progression of C. coli were positives for cdtB however not for cdtA as well as cdtC. This is a significant discovering on the grounds that the absence of either cdtA or cdtC prompts a debilitated creation of CDT

A large portion of the accessible examinations are for the most part worried about the pervasiveness of Campylobacter in poultry as a primary wellspring of human campylobacteriosis. The quantity of studies examining Campylobacter defilement in other meat types is restricted in the writing. In our investigation, we underlined that Campylobacter sullying in meat items other than sheep additionally raise concern, particularly given the high opposition profile of heart, hamburger, and goat Campylobacter confines.

The event of Campylobacter in the examples got from sheeps was marginally higher than in tests of different species. Not a wide range of food showed Campylobacter. spp tainting. liver and kidneies got from shopping, just as meat bought from slaughterhouses were not defiled with Campylobacter microscopic organisms. Campylobacter secludes r from liver only included C. jejuni, while the two species were distinguished at a similar recurrence (half) in goat. meat items. The Chi square test uncovered that C. jejuni secludes were altogether more oftentimes disengaged than C. coli disengages in hamburger meat tests ($p < 0.5$) Although generally little is thought about the harmfulness of Campylobacter spp., these microorganisms have distinctive destructiveness factors (VFs) identified with motility, bond, attack, poison action, insusceptible avoidance, and iron-take-up, among others [2]. Along these lines, while factors, similar to the cadF quality or the iam locus, are engaged with various intrusion steps others, for example, the cytolethal distending poison, a tripartite poison encoded in the cdtA, cdtB, and cdtC qualities which is likewise present in different microorganisms, obstruct the CDC2 kinase, prompting dynamic cell distension which brings about cell passing The least pervasive quality in our examination was recognizable This quality was a factually more regularly distinguished quality in C. coli detaches.

Late examinations unmistakably demonstrate that the meat of ruminants like hamburger might be tainted with Campylobacter and comprise a possible wellspring of campylobacteriosis disease in people. To secure purchasers, there is a requirement for more prominent acknowledgment of sanitation programs "from the homestead to the shopper", further hazard evaluation, and customer training.

We gave an account of Campylobacter defilement of butchered ruminants in significant levels that may speak to expected wellsprings of contamination. Besides, a significant level of protection from ciprofloxacin and antibiotic medication among C. jejuni and C. coli species demonstrate the diminished clinical utility of these anti-infection agents for the treatment of patients. There is additionally a requirement for additional checking of food items according to conceivable transmission of safe Campylobacter to people. The current investigation is the first in Chaharmahal and Bakhtiari Province to survey the recurrence of qualities answerable for destructiveness at various phases of pathogenesis among strains of Campylobacter separated from food of creature starting point, for example, goat , hamburger, pathogenesis among strains of Campylobacter confined from food of creature source, for example, goat , meat, and sheep. In this investigation, the quantity of strains with the key destructiveness factors was huge; be that as it may, contrasts in the recurrence of qualities between various sources and types of Campylobacter were likewise depicted, which ought to be additionally confirmed.

The investigation gives solid relationship among Campylobacter and temperature. Utilizing a scope of factual techniques, the examination recommends that temperature as well as precipitation alone can't clarify the whole occasional variety of *Campylobacteriosis* chance in ruminants Further exploration ought to research if the worldly reliance of the connection between Campylobacter frequency and temperature on the week may be driven by other natural factors, or maybe by an inborn irregularity in the elements of the bacterial populace in the earth or in the zoonotic repository or potential vectors, for example, flies.

In this work, we have exhibited that there is a significant impact of season on the predominance of Campylobacter in a territory that have not been incompletely eradicated. In spite of the fact that there is banter about the items of common sense and cost ramifications of keeping up thorough biosecurity, there is a general agreement inside mainstream researchers that the quantity of positive cases can be and has been diminished by safeguard techniques (1). It might be conceivable to apply improved biosecurity, along the lines of that in routine seasons when the hazard is most prominent, for example, the late spring and pre-winter months.

REFERENCES

1. A. Abamecha, G. Assebe, B. Tafa, and B. Wondafrash, “Prevalence of Thermophilic *Campylobacter* and their Antimicrobial

ResistanceProfileinFoodAnimalsinLareDistrict,NuerZone, Gambella,Ethiopia,"Journal of Drug Research and Development , vol.1,no.2,2015.

2. A. Noormohamed and M. K. Fakhr, "A higher prevalence rate of *Campylobacter* in retail beef livers compared to other beef and pork meat cuts," International Journal of Environmental ResearchandPublicHealth,vol.10,no.5,pp.2058–2068,2013..
3. A.Okunlade,A.O.Ogunleye,F.O.Jeminlehin,andA.T.Ajuwape,"Occurrenceof *Campylobacter* species in beef cattle and local chickens and their antibiotic profiling in Ibadan, Oyo State,Nigeria,"AfricanJournalofMicrobiologyResearch,vol.9, no.22,pp.1473–1479,2015.
4. Adwan K. Fast DNA isolation and PCR protocols for detection of methicillin-resistant staphylococci. Folia Microbiol 2014; 59(1): 5-8.
5. Adwan K. Fast DNA isolation and PCR protocols for detection of methicillin-resistant staphylococci. Folia Microbiol 2014; 59(1): 5-8.
6. Andrzejewska M, Szczepańska B, Śpica D, et al. Trends in the occurrence and characteristics *of Campylobacter jejuni* and *Campylobacter coli* isolates from poultry meat in Northern Poland. Food Control 2015; 51: 190-194
7. Ansari-Lari M, Hosseinzadeh S, Shekarforoush S, et al. Prevalence and risk factors associated with *Campylobacter* infections in broiler flocks in Shiraz, southern Iran. Int J Food Microbiol 2011; 144(3): 475-479. 15
8. Ashrafganjooyi SB, Saedadlei N. Isolation and determine antibiotic susceptibility of *Campylobacter jejuni* in poultry feces in Kerman. Iran J Medic Microbiol 2016; 9(4): 95-98
9. Ashrafganjooyi SB, Saedadlei N. Isolation and determine antibiotic susceptibility of *Campylobacter jejuni* in poultry feces in Kerman. Iran J Medic Microbiol 2016; 9(4): 95-98.
10. Ayaz N, Goncuoglu M, Cakmak O, Erol I. Comparison of *hipO* and *ceuE* gene based on PCR assays for the detection of Campylobacter jejuni. J Clin Microbiol Biochem Technol. 2016; 2 (1):006-008.
11. Bakhshi B, Kalantar M, Rastegar-Lari A, et al. PFGE genotyping and molecular characterization of *Campylobacter spp*. isolated from chicken meat. Iran J Vet Res 2016; 17(3): 177-183
12. Blaser MJ, LaForce FM, Wilson NA, et al. Reservoirs for human campylobacteriosis. J Infect Dis 1980; 141(5): 665-669. 9
13. Brooks GF, Caroll KC, Butel JS, et al. Jawetz, Melnick, & Adelberg's medical microbiology. 25th ed. Columbus, USA: McGraw-Hill Medical. 2006; 342-346
14. D. M. Stone, Y. Chander, A. Z. Bekele et al., "Genotypes, antibiotic resistance, andST-8geneticcloneinCampylobacter isolates from sheep and goats in grenada," Veterinary Medicine International,vol.2014,ArticleID212864,8pages,2014
15. Dabiri H, Aghamohammad S, Goudarzi H, et al. Prevalence and antibiotic susceptibility of *Campylobacter* species isolated from chicken and beef meat. Int J Enteric Pathog 2014; 2(2): e17087
16. Datta S, Niwa H, Itoh K. Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. J Med Microbiol. 2003;52(Pt 4):345-8.
17. Denis M, Refrégier-Petton J, Laisney MJ, et al. Campylobacter contamination in French chicken production from farm to consumers. Use of a PCR assay for detection and identification of Campylobacter jejuni and Camp. coli. J Appl Microbiol 2001; 91(2): 255-267.
18. 18.Duque, B., Daviaud, S., Guillou, S., Haddad, N. and Membre, J.M. (2017) Quantification of *Campylobacter jejuni* contamination on chicken carcasses in France. Food Res. Int., 106: 1077-1085
19. 19.Economou V, Zisides N, Gousia P, et al. Prevalence and antimicrobial profile of Campylobacter isolates from free-range and conventional farming chicken meat during a 6-year survey. Food Control 2015; 56: 161-168.
20. 20.El-Zamkan MA, Hameed KG. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in raw milk and some dairy products. Vet World. 2016;9(10):1147-51. 3
21. 21.Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: A developing country-perspective. Front Microbiol 2016; 7: 1881.
22. 22.Franchin PR, Ogliari PJ, Batista CR. Frequency of thermophilic *Campylobacter* in broiler chickens during industrial processing in a Southern Brazil slaughterhouse. Brit Poult Sci. 2007;48(2):127-32.
23. 23.Friis L, Pin C, Pearson B, et al. In vitro cell culture methods for investigating *Campylobacter* invasion mechanisms. J Microbiol Methds 2005; 61(2): 145-160
24. 24.Ge B, Wang F, Sjölund-Karlsson M, et al. Antimicrobial resistance in *Campylobacter*: susceptibility testing methods and resistance

trends. J Microbiol Methods 2013; 95(1): 57-67.
25. 25.Ge B, White DG, McDermott PF, et al. Antimicrobialresistant *Campylobacter* species from retail raw meats. Appl Environ Microbiol 2003; 69(5): 3005-3007.
26. 26.Ghorbanalizadgan M, Bakhshi B, Kazemnejad Lili A, Najar-Peerayeh S, Nikmanesh B. A molecular survey of *Campylobacter jejuni* and *Campylobacter coli* virulence and diversity. Iran Biomed J. 2014;18(3):15864
27. 27.Ghorbanalizadgan M, Bakhshi B, Najar-Peerayeh S. PCR-RFLP provides discrimination for total flaA sequence analysis in clinical Campylobacter jejuni isolates. Jap J Infect Dis. 2016;69(5):373-7
28. 28.Granić K, Krčar D, Uhitil S, et al. Determination of *Campylobacter. spp* in poultry slaughterhouses and poultry meat. Vet Arhiv 2009; 79(5): 491-497.
29. 29.Hakkinen M, Heiska H, Hänninen ML. Prevalence of *Campylobacter. spp* in cattle in Finland and antimicrobial susceptibilities of bovine Campylobacter jejuni strains. Appl Environ Microbiol 2007; 73(10): 3232-3238.
30. Han X, Zhu D, Lai H, et al. Prevalence, antimicrobial resistance profiling and genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolated from broilers at slaughter in China. Food Control 2016; 69: 160-170
31. 31.Han X, Zhu D, Lai H, et al. Prevalence, antimicrobial resistance profiling and genetic diversity of Campylobacter jejuni and *Campylobacter coli* isolated from broilers at slaughter in China. Food Control 2016; 69: 160-170. 36
32. 32.Hannu T, Mattila L, Rautelin H, et al. Three cases of cardiac complications associated with *Campylobacter jejuni* infection and review of the literature. Eur J Cli Microbiol Infect Dis 2005; 24(9): 619-622
33. 33.Hariharan H, Sharma S, Chikweto A, Matthew V, DeAllie C. Antimicrobial drug resistance as determined by the E-test in *Campylobacter jejuni, C. coli,* and *C. lari* isolates from the ceca of broiler and layer chickens in Grenada. Comp Immunol Microbiol Infect Dis. 2009;32(1):21-8. 23
34. 34.Henry I, Reichardt J, Denis M, Cardinale E. Prevalence and risk factors for *Campylobacter .spp* in chicken broiler flocks in Reunion Island (Indian Ocean). Prev Vet Med. 2011;100(1):64-70
35. 35.Herman L, Heyndrickx M, Grijspeerdt K, Vandekerchove D,Rollier I, De Zutter L. Routes for *Campylobacter* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. Epidemiol Infect.2003;131(3):1169–80. 17
36. 36.Jacobs-Reitsma W, Lyhs U, Wagenaar J. *Campylobacter* in the food supply. In: Nachamkin I, Szymanski CM, Blaser MJ (Eds). *Campylobacter.* 3rd ed. Washington, USA: American Society of Microbiology 2008; 627-644
37. 37.Jamshidi Bassami MR, Farkhondeh T. Isolation and identification of *Campylobacter. spp* and *Campylobacter coli* from poultry carcasses by conventional culture method and multiplex PCR in Mashhad, Iran. Iranian J Vet Res 2008; 9(2): 132-137.
38. 38.Jribi, H., Sellami, H., Hassena, A.B. and Gdoura, R. (2017) Prevalence of putative virulence genes in *Campylobacter* and *Arcobacter* species isolated from poultry and poultry by-products in Tunisia. J. Food Prot., 80(10): 1705-1710.
39. 39.Karikari, A.B., Obiri-Danso, K., Frimpong, E.H. and Krogfelt, K.A. (2017) Antibiotic resistance of *Campylobacter* recovered from faeces and carcasses of healthy livestock. Bio. Med. Res. Int., 2017: 4091856.
40. 40.Kemp R, Leatherbarrow A, Williams N, et al. Prevalence and genetic diversity of *Campylobacter. spp.* in environmental water samples from a 100-squarekilometer predominantly dairy farming area. Appl Environ Microbiol 2005; 71(4): 1876-1882.
41. 41.Khan, J.A., Rathore, R.S., Abulreesh, H.H., Qais, F.A. and Ahmad, I. (2018) Prevalence and antibiotic resistance profiles of *Campylobacter jejuni* isolated from poultry meat and related samples at retail shops in Northern India. Foodborne Pathog. Dis., 15(4): 218-225.
42. 42.Khoshbakht R, Tabatabaei M, Hoseinzadeh S, et al. Prevalence and antibiotic resistance profile of thermophilic *Campylobacter. spp* of slaughtered cattle and sheep in Shiraz, Iran. Vet Res Forum 2016; 7(3): 241-246. 8
43. 43.Khoshbakht R, Tabatabaei M, Hoseinzadeh S, et al. Prevalence and antibiotic resistance profile of thermophilic *Campylobacter. spp* of slaughtered cattle and sheep in Shiraz, Iran. Vet Res Forum 2016; 7(3): 241-246. 8
44. 44.Khoshbakht R, Tabatabaei M, Hosseinzadeh S, Shekarforoush SS, Aski HS. Distribution of nine virulence-associated genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler feces in Shiraz, Southern Iran. Foodborne Pathog Dis. 2013;10(9):764-70. 8
45. 45.Korsak D, Maćkiw E, Rożynek E, et al. Prevalence of *Campylobacter. spp* in retail chicken, turkey, pork, and beef meat in Poland

between 2009 and 2013. J Food Prot 2015; 78(5): 1024-1028.
46. 46.Kramer JM, Frost JA, Bolton FJ, Wareing DR. *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J Food Prot. 2000;63(12):1654-9. 21
47. 47.Little CL, Richardson JF, Owen RJ, et al. *Campylobacter* and Salmonella in raw red meats in the United Kingdom: prevalence, characterization and antimicrobial resistance pattern, 2003–2005. Food Microbiol 2008; 25(3): 538-543.
48. 48.Liu G, Han Y, Li X, et al. Applicability of a rapid method based on immunomagnetic capture-fluorescent PCR assay for *Campylobacter jejuni.* Food Control 2006; 17(7): 527-532. 6
49. 49.Llarena AK, Sivonen K, Hänninen ML. *Campylobacter jejuni* prevalence and hygienic quality of retail bovine ground meat in Finland. Let Appl Microbiol 2014; 58(5): 408-413. 32
50. 50.Macé S, Haddad N, Zagorec M, et al. Influence of measurement and control of microaerobic gaseous atmospheres in methods for *Campylobacter* growth studies. Food Microbiol 2015; 52: 169-176. 3
51. 51.Macé S, Haddad N, Zagorec M, et al. Influence of measurement and control of microaerobic gaseous atmospheres in methods for Campylobacter growth studies. Food Microbiol 2015; 52: 169-176
52. 52.Man SM. The clinical importance of emerging *Campylobacter* species. Nature Rev Gastroenterol Hepatol 2011; 8(12): 669-685.
53. 53.Mpalang, R.K., Boreux, R., Melin, P., Bitiang, K.A.N., Daube, G. and Mol, P.D. (2014) Prevalence of *Campylobacter* among goats and retail goat meat in Congo. J. Infect. Dev. Ctries., 8(2): 168-175.
54. 54.Müller J, Schulze F, Müller W, Hänel I. PCR detection of virulenceassociated genes in Campylobacter jejuni strains with differential ability to invade Caco-2 cells and to colonize the chick gut. Vet Microbiol. 2006;113(1-2):123-9. 15
55. 55.P. B. Gwimi, O. O. Faleke, M. D. Salihu et al., "Prevalence of *Campylobacter* species in fecal samples of pigs and humans from Zuru Kebbi State, Nigeria," International Journal of One Health,vol.1,pp.1–5,2015.
56. 56.Patrick, M E, Christiansen LE, Wainø M, et al. Effects of climate on incidence of *Campylobacter. spp* in humans and prevalence in broiler flocks in Denmark. Appl Environ Microbiol 2004; 70(12): 7474-7480
57. 57.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 JFood Microbiol. 2003;82(3):281-7. 20
58. 58.R.Sharma, K.Tiwari, V.Belmaretal.,"Prevalence and antimicrobial resistance of *Campylobacter* species isolated from backyard chickens in Grenada, West Indies," British Microbiology ResearchJournal,vol.11,no.4,pp.1–8,2016
59. 59.Rahimi E, Ameri M, Alimoradi M, et al. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw camel, beef, and water buffalo meat in Iran. Comp Clin Pathol 2013; 22(3): 467-473.
60. 60.Rahimi E, Ameri M, Kazemeini HR. Prevalence and antimicrobial resistance of *Campylobacter* species isolated from raw camel, beef, lamb, and goat meat in Iran. Foodborne Path Dis 2010; 7(4): 443-447.
61. 61.Rahimi E, Momtaz H, Ameri M, Ghasemian-Safaei H, Ali-Kasemi M. Prevalence and antimicrobial resistance of *Campylobacter* species isolated from chicken carcasses during processing in Iran. Poult Sci. 2010;89(5):1015-20. 18
62. 62.Rozynek E, Dzierzanowska-Fangrat K, Jozwiak P, Popowski J,Korsak D, Dzierzanowska D. Prevalence of potential virulencemarkers in Polish *Campylobacter jejuni* and *Campylobacter coli* isolates obtained from hospitalized children and from chicken carcasses. J Med Microbiol. 2005;54:615–9.
63. 63.Silva DT, Tejada TS, Blum-Menezes D, Dias PA, Timm CD. C*ampylobacter* species isolated from poultry and humans, and their analysis using PFGE in southern Brazil. Int J Food Microbiol. 2016;217:189-94
64. 64.Silva J, Leite D, Fernandes M, Mena C, Gibbs PA, Teixeira P. *Campylobacter. spp* as a foodborne pathogen: a review. Front Microbiol. 2011;2:200. 2
65. 65.Soltan Dallal M, Sanaei M, Taremi M, et al. Prevalence and antimicrobial resistance pattern of thermophilic *Campylobacter spp. (jejuni and coli)* isolated from beef and raw chicken in Tehran. J Zanjan Univ Med Sci 2009; 17(68): 85-92
66. 66.Stanley K, Jones K. Cattle and sheep farms as reservoirs of *Campylobacter*. J Appl Microbiol 2003; 94(s1): 104-113.
67. 67.Stella, S., Soncini, G., Ziino, G., Panebianco, A., Pedonese, F., Nuvoloni, R., Di Gianntale, E., Colavita, G., Alberghini, L. and Giaccone, V. (2017) Prevalence and quantification of

thermophilic *Campylobacter. spp* In Italian retail poultry meat: Analysis of influencing factors. Food Microbiol., 62(1): 232-238.
68. 68.Szczepanska, B., Andrzejewska, M., Spica, D. and Klawe, J.J. (2017) Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. BMC Microbiol., 17(1): 80.
69. 69.T. Lazou, K. Houf, N. Soultos, C. Dovas, and E. Iossifidou, "*Campylobacter* in small ruminants at slaughter: prevalence, pulsotypes and antibiotic resistance," International Journal of FoodMicrobiology,vol.173,pp.54–61,2014
70. 70.Taremi M, Dallal MMS, Gachkar L, et al. Prevalence and antimicrobial resistance of *Campylobacter* isolated from retail raw chicken and beef meat, Tehran, Iran. Int J Food Microbiol 2006; 108(3): 401-403
71. 71.Torralbo A, Borge C, Garcia-Bocanegra I, Meric G, Perea A, Carbonero A. Higher resistance of *Campylobacter coli* compared to Campylobacter jejuni at chicken slaughterhouse. Comp Immunol Microbiol Infect Dis. 2015;39:47-52. 24
72. 72.Trends in the occurrence and characteristics of *Campylobacter jejuni* and *Campylobacter coli* isolates from poultry meat in Northern Poland. Food Control 2015; 51: 190-194.
73. 73.V.Matthew-Belmar,V.A.Amadi,D.Stoneetal.,"Antimicrobial resistance profiles of *Campylobacter jejuni* and *Campylobacter coli* recovered from feces of young healthy domestic pigs in grenada," International Journal Current Microbiology Applied Science,vol.4,no.10,pp.197–206,2015.
74. 74.Van Looveren M, Daube G, De Zutter L, et al. Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. J Antimicrob Chemother 2001; 48(2): 235-240. 39
75. 75.Whyte P, McGill K, Cowley D, et al. Occurrence of *Campylobacter* in retail foods in Ireland. Int J Food Microbiol 2004; 95(2), 111-118.
76. 76.Wikler MA. Performance standards for antimicrobial susceptibility testing: 16th informational supplement. Wayne, USA: Clinical and Laboratory Standards Institute 2006; 32-38.
77. 77.Zendehbad B, Arian AA, Alipour A. Identification and antimicrobial resistance of *Campylobacter* species isolated from poultry meat in Khorasan province, Iran. Food Control 2013; 32(2): 724-727
78. 78.Zhang T, Luo Q, Chen Y, Li T, Wen G, Zhang R, et al. Molecular epidemiology, virulence determinants and antimicrobial resistance of *Campylobacter* spreading in retail chicken meat in Central China. Gut Pathog. 2016;8:4