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Original Article

Prevalence and antibiotic resistance pattern of *Campylobacter* species isolated from raw cow milk in Urmia, Iran

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ABSTRACT

Campylobacter species are the most common bacterial cause of diarrhea in humans. The present study investigated the prevalence and antibiotic resistance of *Campylobacter* spp. isolated from raw milk in Urmia, Iran. Eighty raw cow milk samples were randomly collected from traditional dairy retailers in different regions of Urmia by sterile conditions in 2018. First, the samples were enriched in supplemented Preston broth, and then they were streaked onto supplemented *Campylobacter* agar and incubated at 42 °C for 48 h in microaerophilic conditions. Biochemical tests, such as hippurate hydrolysis and susceptibility or resistance to nalidixic acid, were performed to identify the species of isolates. Antibiotic sensitivity test on isolates was performed by the Kirby-Bauer disc diffusion method. Thirteen samples (16.25%) were contaminated with *Campylobacter* spp. The prevalence of *Campylobacter jejuni* (13.75%) was higher than *Campylobacter coli* isolates (2.5%). The *Campylobacter* isolates showed high resistance against tetracycline (100%), co-trimoxazole (84%), ampicillin, ceftriaxone, and chloramphenicol (69.2%) while exhibiting moderate resistance to ciprofloxacin and nitrofurantoin (46.2%) and low resistance to gentamicin (30.8%). Moreover, nine isolates (69.2%) showed multi-drug resistance (MDR). It can be concluded the prevalence of *Campylobacter* spp. and their MDR strains in distributed raw cow milk in Urmia is high. It is recommended to improve animal health and milk hygiene, prevent the overuse of antibiotics in dairy farms, and pasteurize milk.

بررسی شیوع و الگوی مقاومت آنتی بیوتیکی گونه‌های *کمپیلوباکتر* جدا شده از شیر خام گاو در ارومیه، ایران

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چکیده

گونه‌های *کمپیلوباکتر* شایع‌ترین عامل اسهال باکتریایی در انسان می‌باشند. در تحقیق حاضر شیوع و مقاومت آنتی‌بیوتیکی گونه‌های *کمپیلوباکتر* جدا شده از شیر خام شهرستان ارومیه بررسی‌شدند. ۸۰ نمونه شیر خام گاو به‌صورت تصادفی و با شرایط سترون از خرده فروشی‌های لبنیات سنتی مناطق مختلف ارومیه در سال ۱۳۹۷ جمع‌آوری شدند. نمونه‌ها، ابتدا در آبگوشت پرستون تکمیل‌شده، غنی‌سازی و سپس در آگار تکمیل شده *کمپیلوباکتر* کشت و در دمای ۴۲ درجه‌سلسیوس به‌مدت ۴۸ ساعت در شرایط میکروآیروفیل گرمخانه‌گذاری شدند. آزمایش‌های بیوشیمیایی ازجمله هیدرولیز هیپورات و حساسیت یا مقاومت به نالیدیکسیک اسید برای شناسایی گونه‌ی جدایه‌ها انجام شدند. آزمایش حساسیت آنتی‌بیوتیکی روی جدایه‌ها به‌روش انتشار دیسک کربی–بائر انجام شد. ۱۳ نمونه (۱۶/۲۵ درصد) آلوده به گونه‌های *کمپیلوباکتر* بودند. شیوع جدایه‌های *کمپیلوباکتر ججونای* (۱۳/۷۵ درصد) بالاتر از *کمپیلوباکتر کولای* (۲/۵ درصد) بود. جدایه‌های *کمپیلوباکتر* مقاومت بالا در برابر تتراسایکلین (۱۰۰ درصد)، کوتریموکسازول (۸۴ درصد)، آمپی‌سیلین، سفتریاکسون و کلرامفنیکل (۶۹/۲ درصد) نشان دادند درحالی‌که آنها مقاومت متوسط به سیپروفلوکساسین و نیتروفورانتوئین (۴۶/۲ درصد) و مقاومت پایین به جنتامایسین (۳۰/۸ درصد) نشان دادند. هم‌چنین ۹ جدایه (۶۹/۲ درصد) مقاومت چند دارویی (MDR) نشان دادند. می‌توان نتیجه‌گیری نمود که شیوع گونه‌های *کمپیلوباکتر* و سویه‌های MDR آنها در شیر خام گاو توزیعی ارومیه بالا می‌باشد. ارتقا سلامت دام و بهداشت شیر، جلوگیری از مصرف بیش از حد آنتی‌بیوتیک‌ها در مزارع گاوان شیری و پاستوریزاسیون شیر پیشنهاد می‌گردد.

واژه های کلیدی: شیرخام، گونه‌های کمپیلوباکتر، الگوی مقاومت آنتی بیوتیکی، ارومیه، ایران

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INTRODUCTION

Campylobacter jejuni and *Campylobacter coli* are recognized as the leading cause of human bacterial diarrhea worldwide [1]. *Campylobacter* species are small, motile, non-spore-forming, curved, or spiral gram-negative rods [2,3] which are microaerophilic organisms and grow best in an atmosphere containing 3-6% oxygen [4,5]. In addition, *Campylobacter* spp. are known as capnophilic organisms, and their growth is enhanced in the presence of 2–10% CO2 [4,5]. Although *Campylobacter* spp. grow at 37 °C, the optimum temperature for the multiplication of thermophilic species, including *C. jejuni*, *C. coli*, and *C. lari*, is 42 °C [2,3,5]. The thermophilic Campylobacters can be differentiated based on nalidixic acid susceptibility and hippurate hydrolysis [3]. The infectious dose of *C. jejuni* is low, and it can lead to infection by surviving in small numbers (50 to 500 organisms) in contaminated foods [1,3,5]. *Campylobacter* spp. can cause gastrointestinal or extraintestinal infections [1,3]. Campylobacter enteritis typically occurs in healthy individuals by ingestion of Campylobacter-contaminated food or water or occupational contact with Campylobacter-infected animals [1,3,5]. Extraintestinal infection, including bacteremia and various organ infections, usually occurs following the systemic spread of Campylobacter in immunocompromised individuals [1,3]. Milk is a nutrient medium for the growth of microorganisms [6]. Microbial contamination of raw milk results from three leading sources: inside of the udder (systemic infections and mastitis), outside of the udder (feces), and through the milk contact surfaces (milker's hands, milking machine, and storage equipment) [7]. In recent years raw milk has been a common source of *Campylobacter* spp. [3]. These organisms are commonly found as commensal organisms in the gastrointestinal tracts of cattle, and their presence in raw milk usually results from fecal contamination during the milking stages [8]. A few publications have reported the direct excretion of *Campylobacter* into milk in mastitis [8]. Campylobacteriosis outbreaks related to raw milk consumption have been reported worldwide [8-11]. However, these outbreaks have significantly decreased with the pasteurization of milk and raised public awareness in developed countries [3]. *Campylobacter* infections are self-limiting, and antibiotic treatment is not usually required [12]. However, antibiotic treatment is necessary under certain clinical conditions, such as severe cases, infections in immunocompromised patients, and long-term infections [12]. Currently, macrolide antibiotics, such as erythromycin and azithromycin, and fluoroquinolone antibiotics, such as ciprofloxacin, are considered as choice drugs for treating campylobacteriosis [12,13]. Furthermore, tetracycline and gentamicin are used as alternative antibiotics in *Campylobacter* infections [12,13]. In developing countries, the inappropriate use of antibiotics in the treatment of human and animal infections, the lack of strict regulations in this field, and allowing to use of antibiotics as growth enhancers in food animal farms lead to the emergence of antibiotic-resistant bacterial strains, especially multi-drug resistant (MDR) strains [12]. The bacterial strains resistant to three or more antibiotic classes are frequently known as MDR strains [14]. *Campylobacter* spp. have a high capacity to transfer genetic components [15]. This characteristic allows antibiotic-resistance genes to be transferred easily [15]. In recent years, the concern about Campylobacter infections has grown due to the increase of multidrug-resistant isolates in patients with enteritis and diarrhea in developing and developed countries [16]. Currently, the resistance of *Campylobacter* spp. to penicillin, cephalosporin, and sulfonamides has been observed [13]. The present study investigates the prevalence and antibiotic resistance pattern of *Campylobacter* spp. isolated from distributed raw cow milk in Urmia, Iran.

MATERIALS AND METHODS

Sampling

The current research is a cross-sectional descriptive study. Eighty raw cow milk samples were randomly gathered from traditional dairy retailers in different regions of Urmia, Iran, from October to December 2018.

Isolation and Identification of Campylobacter spp.

The pH of the samples was adjusted to 7.50 ± 0.20 with a digital pH meter (Oriba, Japan) using a sterile 1 N NaOH (1.06462, Merck KGaA, Darmstadt, Germany) solution. 50 ml of the pH-adjusted samples were centrifuged (Universal Centrifuge Premium 20000, Pole Ideal Tajhiz Co., Tehran, Iran) at 12٠٠٠ g for 40 min. Then, the supernatant was discarded, and the sediment was suspended in 10 ml of Preston enrichment broth base (M899, Himedia laboratories, Mumbai, India) containing *Campylobacter* supplement IV (FD042, Himedia Laboratories), 5% (v/v) sterile defibrinated sheep blood, and then transferred to the bottle containing 90 ml of Preston enrichment broth with the same characteristic. The tested bottles were incubated at 42 °C for 24 h in a microaerophilic environment using a sealed jar with CampyGen gas-generating envelope (CN025A, Oxoid, UK). The enriched samples were streaked onto *Campylobacter* agar (M994, Himedia Laboratories) containing *Campylobacter* supplement I (FD006, Himedia Laboratories), 5% (v/v) sterile defibrinated sheep blood, and incubated at 42 °C for 48 h under the same condition. To identify the species of isolates, typical colonies (non-hemolytic, flat, gray, round with a mucoid appearance) were subjected to growth at 25 °C, catalase, nitrate reduction, H2S production, hippurate hydrolysis, and susceptibility or resistance to nalidixic acid and cephalothin tests [17-21].

Antimicrobial Susceptibility Testing

The antimicrobial sensitivity test on isolates was carried out using the Kirby-Bauer disk diffusion method [22-24]. First, the isolates sub-cultured on blood agar base (M073, Himedia Laboratories) containing 5% (v/v) sterile defibrinated sheep blood. Then, some colonies were suspended in sterile saline, and the turbidity of the suspension was adjusted visually with 0.5 McFarland standard solution (approximately 1.5 x 10^8 CFU/ml). The inoculum was streaked with swabs thoroughly onto Mueller-Hinton agar (MV1084, Himedia Laboratories) supplemented with 5% (v/v) sterile defibrinated sheep blood and then the desired antibiotic disks (Padtan Teb, Tehran, Iran), including ampicillin (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), co-trimoxazole (trimethoprim and sulphamethoxazole) (1.25/23.75 µg), gentamicin (10 µg), nitrofurantoin (300 µg) and tetracycline (30 µg), were placed on the inoculated agar. The tested plates were incubated at 42 ˚C for 24 h in a microaerophilic condition. The growth inhibition zone of each disk was measured (mm), and the results were interpreted by the clinical laboratory standard institute (CLSI) criteria [22].

RESULTS

Thirteen samples (16.25%) of raw milk were contaminated with *Campylobacter* spp. The frequency of samples contaminated with *C. jejuni* (13.75%) was higher than the samples contaminated with *C. coli* (2.5%) (Fig. 1). As shown in Table 1, *Campylobacter* isolates exhibited high resistance against tetracycline (100%), co-trimoxazole (84.6%), ampicillin, ceftriaxone, and chloramphenicol (69.2%). Also, the isolates showed moderate resistance against ciprofloxacin and nitrofurantoin (46.2%) and low resistance to gentamicin (30.8%) (Table 1). Moreover, resistance to one or more antibiotic classes was observed in all isolates (Table 2). The MDR strains of *Campylobacter* were found in nine isolates (69.2%) (Table 2). In addition, the frequency of the MDR strains in *C. coli* isolates (100%) was more than in *C. jejuni* isolates (72.7%) (Table 2).

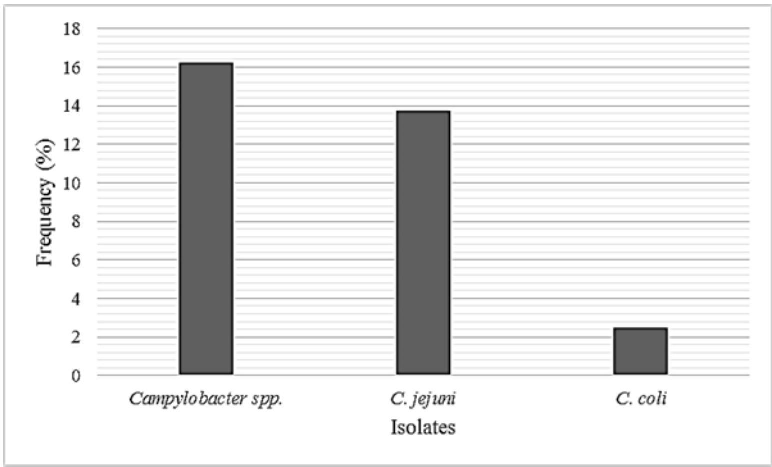

Figure 1. Prevalence of Campylobacter spp. isolated from raw milk in Urmia, Iran.

Table 1. Antibiotic susceptibility profile of *Campylobacter* spp. isolated from raw milk

Antibiotics	***Campylobacter* spp. (N=13)**			***C. jejuni* (N=11)**			***C. coli* (N=2)**		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Ampicillin	0 (0.0%)	4 (30.8%)	9 (69.2%)	0 (0.0%)	3 (27%)	8 (73%)	0 (0.0%)	1 (50%)	1 (50%)
Ceftriaxone	2 (15.4%)	2 (15.4%)	9 (69.2%)	2 (18%)	1 (9%)	8 (73%)	0 (0.0%)	1 (50%)	1 (50%)
Chloramphenicol	0 (0.0%)	4 (30.8%)	9 (69.2%)	0 (0.0%)	4 (36.4%)	7 (63.6%)	0 (0.0%)	0 (0.0%)	2 (100%)
Ciprofloxacin	6 (46.2%)	1 (7.6%)	6 (46.2%)	6 (54.5%)	1 (9.1%)	4 (36/6%)	0 (0.0%)	0 (0.0%)	2 (100%)
Co-trimoxazole	0 (0.0%)	2 (15.4%)	11 (84.6%)	0 (0.0%)	2 (18%)	9 (82%)	0 (0.0%)	0 (0.0%)	2 (100%)
Gentamicin	6 (46.2%)	3 (23%)	4 (30.8%)	4 (36.4%)	3 (27.2%)	4 (36.4%)	2 (100%)	0 (0.0%)	0 (0.0%)
Nitrofurantoin	7 (53.8%)	0 (0.0%)	6 (46.2%)	7 (63.6%)	0 (0.0%)	4 (36.4%)	0 (0.0%)	0 (0.0%)	2 (100%)
Tetracycline	0 (0.0%)	0 (0.0%)	13 (100%)	0 (0.0%)	0 (0.0%)	11 (100%)	0 (0.0%)	0 (0.0%)	2 (100%)

N: number; S: susceptible; I: intermediate; R: resistant

Table 2. Antibiotic resistance pattern and frequency of multi-drug resistant strains in Campylobacter spp. isolated from raw milk

Isolates	**Antibiotic resistance**								MDR
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	
***Campylobacter* spp. (N=13)**	2 (15.4%)	2 (15.4%)	0 (0.0%)	0 (0.0%)	3 (23.1%)	0 (0.0%)	2 (15.4%)	4 (30.7%)	9 (69.2%)
***C. jejuni* (N=11)**	2 (18.2%)	1 (9.1%)	0 (0.0%)	1 (9.1%)	3 (27.3%)	0 (0.0%)	0 (0.0%)	4 (36.3%)	8 (72.7%)
***C. coli* (N=2)**	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	2 (100%)

N: number; R1: resistance to one; R2: resistance to two; R3: resistance to three; R4: resistance to four; R5: resistance to five; R6: resistance to six; R7: resistance to seven; R8: resistance to eight antibiotics; MDR: multi-drug resistance (resistance to three or more antibiotics)

DISCUSSION

According to the results, the prevalence rate of *Campylobacter* spp. in distributed raw cow milk in Urmia was 16.25%. The frequency of *C. jejuni* isolates (13.75%) was higher than *C. coli* isolates (2.5%). The existance of *Campylobacter* spp. in raw milk primarily occurs after fecal contamination [8,25,26]. Dairy cows are known as Campylobacter reservoirs [25]. National Animal Health Monitoring System (NAHMS) surveys in the United States have detected *Campylobacter* spp. on dairy farms, especially in feces samples [25]. Furthermore, *Campylobacter* organisms have been isolated from bulk tank milk (BTM) in dairy farms [26,27]. It has been shown that *Campylobacter* spp. are occasionally implicated in mastitis [8,28,29]. Taylor *et al*. reviewed 262 campylobacteriosis outbreaks in the United States from 1997 to 2008. They reported that 225 outbreaks (86%) were foodborne, and dairy products and poultry meat were implicated in 65 (29%) and 25 (11%) outbreaks, respectively [30]. In a review, the global prevalence of *Campylobacter* spp. in raw milk was reported by Taghizadeh *et al*. at approximately 4% [31]. In this review, the lowest prevalence was perceived in Europe (1%), and the highest prevalence was observed in Oceania (9%) and Asia (7%), followed by Africa (6%) and America (5%) [31]. Moreover, the contamination rate of the raw cow milk samples with *Campylobacter* spp. in different regions of Iran has been studied. In a survey in Mazandaran province, the contamination rate of raw cow milk samples from traditional dairy retailers with *C. jejuni* was reported by Khoshbakht *et al*. at 7% [32]. In another survey in Mazandaran province, the contamination rate of raw milk in a dairy cattle farm with *Campylobacter* spp., *C. jejuni*, and *C. coli* was obtained by Raeisi *et al*. at 8.75%, 6.25%, and 2.5%, respectively [33]. Furthermore, in a survey in Amol city, the prevalence rate of *C. jejuni* in raw cow milk samples from milk collection centers was reported by Dabiri *et al*. at 13.88% [34]. Kazemini *et al*. obtained the prevalence rate of *C. jejuni* in raw milk samples in dairy bovine herds in Isfahan city at 2.5 % [35]. Khanzadi *et al*. also reported the contamination rate of BTM samples from dairy cattle farms in Mashhad city with *C. jejuni* at 8% [36]. Therefore, as observed, the prevalence of *Campylobacter* spp. in raw cow milk in various regions of Iran and the world is different. These differences can be due to changes in geographical location, season, sample type, sample size, sampling methods, isolation methods, farming system, milking method, and sanitary conditions in dairy farms [31]. In the present study, similar to the findings of other researchers in the world and Iran [31-34], *C. jejuni* was the most common isolate from raw cow milk. The prevalence rate of *C. jejuni* in our survey was consistent with the results of Dabiri *et al*. [34]. Compared to the findings of Khoshbakht *et al*. [32], Raeisi *et al*. [33], Kazemini *et al*. [35], Khanzadi *et al*. [36], the global prevalence of *Campylobacter* spp. in milk [31] was high. It can be attributed to traditional cattle farms with manual milking systems and poor animal hygiene in the region. Therefore, it is recommended to improve the hygienic status of dairy farms, especially traditional types, by using livestock health and milk hygiene standards. However, even with strict implementation of hygiene measures in dairy farms, low levels of Campylobacter contamination may be observed. Thus, avoiding the consumption of raw milk and milk pasteurization is suggested. Fluoroquinolones, particulaly ciprofloxacin, are commonly used antibiotics for treating *Campylobacter* infections in human [12,13]. A tendency to the expansion of fluoroquinolone-resistant *Campylobacter* strains from human and animal origin in the United States and Canada (19-47%), Europe (17-99%), Africa, and Asia (>80%) has been reported [37]. The rapid emergence of fluoroquinolone-resistant *Campylobacter* strains worldwide may be partially attributed to the extensive use of fluoroquinolones in veterinary medicine, especially in poultry farms [12]. In a review, the resistance of *Campylobacter* isolates from humans and animal samples to quinolone and fluoroquinolone antibiotics, including nalidixic acid, ciprofloxacin, enrofloxacin, and ofloxacin, was reported by Khademi and Sahebkar at 0% to 87.3% [13]. The difference in the prevalence rate appears to be related to the type of sample (human or animal origin) and the low or high use of these antibiotics in human or animal infections. Based on our results, *Campylobacter* isolates showed moderate resistance to ciprofloxacin (46.2%). This finding may be due to the low usage of fluoroquinolone antibiotics in dairy farms. The resistance to ciprofloxacin is primarily mediated through point mutations in the quinolone resistance-determining region (QRDA) of DNA gyrase A (GyrA) [37]. In addition to fluoroquinolones and macrolides, tetracycline and gentamicin are used as alternative antibiotics in human campylobacteriosis [13]. Tetracycline is extensively used in veterinary medicine. According to our results, *Campylobacter* isolates exhibited high resistance to tetracycline (100%). This finding is consistent with the results of other researchers in Iran and other countries [20,21,25,38-42]. The *tet*(O) gene is responsible for tetracycline resistance in Campylobacter and is widely found in animal isolates [37]. In most strains, the *tet*(O) gene is encoded by a plasmid, but in some isolates, there is a chromosomal-encoded copy of the gene [37]. In the present research, *Campylobacter* isolates showed low resistance to gentamicin (30.8%). This finding is similar to the results of other researchers [20,21,33,43]. The use of aminoglycoside antibiotics in food animal farms is limited due to toxicity and long withdrawal period [44]. The low resistance of isolates to gentamicin may be associated with the restricted usage of this antibiotic in food animal farms. Gentamicin resistance in *Campylobacter* spp. is mainly caused by aminoglycoside modifying enzyme [37]. In our study, *Campylobacter* isolates showed high resistance to ampicillin and ceftriaxone (69.2%). The high resistance of *Campylobacter* isolates to beta-lactam antibiotics may be due to their excessive use in dairy farms, especially in mastitis. Intrinsic resistance and beta-lactamase production are two leading mechanisms in the resistance of *Campylobacter* spp. to beta-lactam antibiotics [37]. According to the results, *Campylobacter* isolates exhibited moderate resistance to nitrofurantoin (46.2%). Also, low to moderate resistance to nitrofurantoin in human or animal isolates of *Campylobacter* has been reported [45,46]. It may be related to the limited usage of nitrofurantoin in medicine and veterinary medicine. Interestingly, *C. jejuni and C. coli* are intrinsically resistant to trimethoprim and sulfamethoxazole [48,49]. Therefore, the high resistance of *Campylobacter* isolates to co-trimoxazole (84.6%) is justified. Although some studies have shown that the prevalence of chloramphenicol-resistant *Campylobacter* strains is very low [43,49], in our research, *Campylobacter* isolates showed high resistance to chloramphenicol (69.2%). Resistance to chloramphenicol in *Campylobacter* spp. is achieved with the *cat* gene [48]. The *cat* gene is carried on a plasmid and encodes the chloramphenicol acetyltransferase (CAT) enzyme and prevents the binding of the chloramphenicol to ribosomes [48]. Furthermore, the *cat* gene has been identified in *C. coli* [50]. To account for the adverse findings of the current research, more studies are required on the identification of *cat* genes in *Campylobacter* isolates. Also, the MDR strains of *Campylobacter* were found at a high level in the present study (69.2%). This finding is consistent with the results of other researchers in Iran [20,21,33,51-53]. The emerge and spread of MDR strains of *Campylobacter* is a public health issue worldwide. The high rate of MDR strains may be attributed to the overuse of antibiotics in dairy farms and the lack of strict regulations in this field. The existence of an efflux system causes resistance to a broad range of antibiotics in *Campylobacter* spp. In the current research, the frequency of MDR strains in *C. coli* isolates was higher than in *C. jejuni* isolates. Other researchers have also found the same findings [33]. It appears that receiving antibiotic resistance genes in *C. coli* occurs better than in *C. jejuni*; the mutation of the target genes in *C. coli* happens faster than in *C. jejuni* [51,52]. Therefore, the formulation and implementation of strict regulations about the usage of antibiotics in food animal farms and the appropriate use of antibiotics in veterinary medicine and medicine are recommended.

CONCLUSION

The prevalence of *Campylobacter* spp. in distributed raw cow milk samples in Urmia was high. *C. jejuni* was the most frequent

Campylobacter isolates. Improvement of animal health and milk hygiene in dairy cattle farms of the region, especially traditional type, is highly recommended. Also, *Campylobacter* isolates showed high resistance to tetracycline, moderate resistance to ciprofloxacin, and low resistance to gentamicin. The frequency of MDR strains in *Campylobacter* isolates in raw cow milk samples was high. Limited and appropriate usage of antibiotics in food animal farms is suggested. Considering that sampling was restricted to only a few months in the current research, it is recommended to conduct a comprehensive study with an annual sampling plan in the region.

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ETHICS

Approved.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Razavilar V. Pathogenic Microorganisms in Foods and Epidemiology of Foodborne Intoxications. 4th ed. Tehran University Publications Institute, 2016; 103-10. [In Persian]

[2] Franco DA, Williams CE. *Campylobacter jejuni*. In: Hu YH, Pierson MD, Gorham JR (Eds). Foodborne Disease Handbook Volume 1: Bacterial Pathogens. 2nd ed. Marcel Dekker, Inc., 2001; 83-106.

[3] Hu L, Kopecko Dj. *Campylobacter* species. In: Miliotis MD, Bier JW (Eds). International Handbook of Foodborne Pathogens. 1st ed. Marcel Dekker, Inc., 2003; 188-205.

[4] Jay JM, Loessner MJ, Golden DA. Modern Food Microbiology. 7th ed. Springer Science Business Media, Inc., 2005; 661-8.

[5] Rowe MT, Madden, RH. *Campylobacter*. In: Batt CA, Tortorello LM (Eds). Encyclopedia of Food Microbiology. Academic press, 2014; 348-53.

[6] Razavi-Rohani SM, Moradi M. Milk Hygiene. 1st ed. Urmia University Publications, 2016; 255-75. [In Persian]

[7] Zastempowska E, Grajewski J, Twarużek, M. Food-borne pathogens and contaminants in raw milk –a review. Annual Animal Science, 2016; 16(3): 623-39. **doi:10.1515/aoas-2015-0089**

[8] Heuvelink AE, Heerwaarden CV, Zwartkruis-Nahuis A, Tilburg JJHC, Bos MH, Heilmann, FGC, et al. Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. International Journal of Food Microbiology, 2009; 134: 70-74. **doi: 10.1016/j.ijfoodmicro.2008.12.026**

[9] Kenyon J, Inns T, Aird H, Swift C, Astbury J, Forester E, et al. *Campylobacter* outbreak associated with raw drinking milk, North West England, 2016. Epidemiology and Infection, 2020; 148, e13, 1-6. **doi:10.1017/S0950268820000096**

[10]. Fahey T, Morgan D, Gunneburg C, Adak GK, Majid F, Kaczmarski E. An outbreak of *Campylobacter jejuni* enteritis associated with failed milk pasteurization. Journal of Infection, 1995; 31: 137-43. **doi:10.1016/S0163-4453(95)92160-5**

[11] Morgan D, Gunneberg C, Gunnell D, Healing, TD, Lamerton, S, Soltanpoor, N, et al. An outbreak of *Campylobacter* infection associated with the consumption of unpasteurized milk at a large festival in England. European Journal of Epidemiology, 1994; 10: 581-5.

[12] Osaili TM, Alaboudi AR. Antimicrobial Resistance of *Campylobacter* spp. In: Singh OM (Eds). Foodborne Pathogens and Antibiotic Resistance. 1st ed. John Wiley & Sons, Inc., 2017; 417- 30.

[13] Khademi F, Sahebkar, A. Prevalence of fluoroquinolone-resistant *Campylobacter* species in Iran: a systematic review and meta-analysis. Hindawi International

Journal of Microbiology, 2020; 2029. **doi:10.1155/2020/8868197**

[14] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske, CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology Infection, 2012; 18: 268-81. **doi:10.1111/j.1469-0691.2011.03570.x**

[15] Neogi SB, Islam MdM, Islam SKS, Akhter AHMT, Skder MdMH, Yamasaki S, et al. Risk of multi-drug resistant *Campylobacter* spp. and residual antimicrobials at poultry farms and live bird markets in Bangladesh. BMC Infectious Diseases, 2020; 20:278. **doi:10.1186/s12879-020-05006-6**

[16] Du J, Luo J, Huang J, Wang C, Li M, Wang B, et al. Emergence of genetic diversity and multi-drug resistant *Campylobacter jejuni* from wild birds in Beijing, China. Frontiers in Microbiology, 2019; 10: 2433. **doi: 10.3389/fmicb.2019.02433**

[17] Food and Drug Administration (FDA). Bacteriological Analytical Manual (BAM) Chapter 7: *Campylobacter*. U.S. Food and Drug administration. Available at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-7-campylobacter. Accessed 08/03/2021.

[18] Stern NJ, Patton CM, Doyle MP, Park CE, McCardell BA. *Campylobacter*. In: Vanderzant C, Splittstoesser DF (Eds). Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. American Public Health Association, 1992; 475-89.

[19] Markey B, Leonard F, Archambault M, Cullinane A, Magurie D. Clinical Veterinary Microbiology. 2nd ed. Elsevier Ltd, 2013; 335-44.

[20] Maktabi S, Ghorbanpoor M, Hossaini M, Motavalibashi A. Detection of multi-antibiotic resistant *Campylobacter coli* and *Campylobacter jejuni* in beef, mutton, chicken and water buffalo meat in Ahvaz, Iran. Veterinary Research Forum 2019; 10 (1): 37-42. **doi:10.30466/vrf.2019.34310**

[21] 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. Poultry Science, 2010; 89:1015-20. **doi:10.3382/ps.2009-00090**

[22] CLSI. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

[23] Ortez JH. Manual of Antimicrobial Susceptibility Testing. In: Disk Diffusion Testing. Ed, Coyle MB, 1st ed. American Society for Microbiology, 2005; 39-52.

[24] Ge B, Wang F, Sjölund-Karlsson M, McDermott PF. Antimicrobial resistance in *Campylobacter*: susceptibility testing methods and resistance trends. Journal of Microbiology Methods, 2013; 95(1): 57-67. **doi: 10.1016/j.mimet.2013.06.021**

[25] Del Collo LP, Karns JS, Biswas D, Lombard JE, Haley BJ, Kristensen RC, et al. Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter* spp. in bulk tank milk and milk filters from US dairies. Journal of Dairy Science, 2016; 100:3470-79. **doi:10.3168/jds.2016-12084**

[26] Bianchini V, Borella L, Benedetti V, Parisi A, Miccolupo A, Santoro E, et al. Prevalence in bulk tank milk and epidemiology of *Campylobacter jejuni* in dairy herds in Northern Italy. Applied and Environmental Microbiology, 2014; 80:1832-37. **doi: 10.1128/AEM.03784-13**

[27] Halbert L W, Kaneene J B, Ruegg PL, Warnick LD, Wells SJ, Mansfield LS, et al. Evaluation of antimicrobial susceptibility patterns in *Campylobacter* spp. isolated from dairy cattle and farms managed organically and conventionally in the midwestern and northeastern United States. Journal of the American Veterinary Medicine Association, 2006; 228:1074-81. **doi: 10.2460/javma.228.7.1074**

[28] Morgan G, Chadwick P, Lander KP, Gill KPW. *Campylobacter jejuni* mastitis in a cow: A zoonosis-related incident. Veterinary Record, 1985; 116: 111.

[29] Orr KE, Lightfoot NF, Sisson PR, Harkis BA, Tweddle JL, Boyd P, et al. Direct milk excretion of *Campylobacter jejuni* in a dairy cow causing cases of human enteritis. Epidemiology & Infection, 1995; 114:15-24. **doi: 10.1017/s0950268800051876**

[30] Taylor EV, Herman KM, Ailes EC, Fitzgerald C, Yoder, JS, Mahon BE, et al. Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. Epidemiology & Infection, 2013; 141: 987-96. **doi: 10.1017/S0950268812001744**

[31] Taghizadeh M, Nematollahi A, Bashiry M, Javanmardi F, Mousavi M, Hosseini H. The global prevalence of *Campylobacter* spp. in milk: A systematic review and meta-analysis. International Dairy Journal, 2022; 133: 105423**.** **doi: 10.1016/j.idairyj.2022.105423**

[32] Khoshbakht R, Kazemeini H, Panahi Z. Molecular detection of *Campylobacter* species and *Salmonella* spp. in cattle raw milk specimens in Mazandaran province. Iranian Journal of Food Science and Technology, 2020; 125(19): 101-8 [In Persian]. **doi:10.22034/FSCT.19.125.101**

[33] Raeisi M, Khoshbakht R, Ghaemi EA, Bayani M, Hashemi M, Seyedghasemi NS, et al. Antimicrobial resistance and virulence-associated genes of *Campylobacter* spp. isolated from raw milk, fish, poultry, and red meat. Microbial Drug Resistance, 2017; 23(7): 925-33. **doi:10.1089/mdr.2016.0183**

[34] Dabiri A, Rouhi S, Nouri B, Zaboli F. The prevalence of *Campylobacter* genus and *Campylobacter jejuni* species in raw milk collected from Amol by multiplex-polymerase chain reaction. Journal of Fasa University of Medical Sciences, 2016; 5(4): 516-525. [In Persian]. **dor: 20.1001.1.22285105.2016.5.4.5.0**

[35] Kazemeini H, Valizade Y, Parsaei P, Nozarpour, N, Rahimi E. Prevalence of *Campylobacter* species in raw bovine milk in Isfahan, Iran. Middle-East Journal of Scientific Research, 2011; 10 (5): 664-6.

[36] Khanzadi S, Jamshidi A, Soltaninejad V, Khajenasiri S. Isolation and identification of *Campylobacter jejuni* from bulk tank milk in Mashhad-Iran. World Applied Sciences Journal, 2010; 9(6):638-43.

[37] Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM, Zhang Q. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiology, 2009; 4(2): 189-200. **doi:10.2217/17460913.4.2.189**

[38] Al-Natour MQ, Alaboudi AR, Osaili TM, Obaidat MM. Resistance of *Campylobacter jejuni* isolated from layer farms in northern Jordan using microbroth dilution and disc diffusion techniques. Journal of Food Science, 2016; 81(7): M1749-53. **doi: 10.1111/1750-3841.13363**

[39] Piddock LJ, Griggs D, Johnson MM, Ricci V, Elviss NC, Williams LK, et al. Persistence of *Campylobacter* species, strain types, antibiotic resistance and mechanisms of tetracycline resistance in poultry flocks treated with chlortetracycline. Journal of Antimicrobial Chemotherapy, 2008; 62 (2): 303-15. **doi: 10.1093/jac/dkn190**

[40] Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA. Prevalence of Arcobacter and *Campylobacter* on broiler carcasses during processing. International Journal of Food Microbiology, 2005; 113 (1): 16-22. **doi: 10.1016/j.ijfoodmicro.2006.06.033**

[41] Itoh T, Takahashi M, Kai A, Takano I, Saito K, Ohashi M. Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated from human and animals in Japan. Journal of the Japanese Association for Infectious Diseases, 1984; 58 (11): 1206-12.

[42] Pratt A, Korolik V. Tetracycline resistance of Australian *Campylobacter jejuni* and *Campylobacter coli* isolates.

Journal of Antimicrobial Chemotherapy, 2005; 55 (4): 452-60.
doi: 10.1093/jac/dki040

[43] Deckert A, Valdivieso-Garcia A, Reid-Smith R, Tamblyn S, Seliske P, Irwin R, et al. Prevalence and antimicrobial resistance in *Campylobacter* spp. isolated from retail chicken in two health units in Ontario. Journal of Food Protection, 2010; 73(7)*:*1317–1324.
doi: 10.4315/0362-028X-73.7.1317

[44] Dowling PM. Aminoglycosides and aminocyclitols, antimicrobial therapy in veterinary medicine. John Wiley & Sons, Inc., 2013; 233-55.

[45] Adekunle OC, Onilude AA. Antimicrobial resistance and plasmid profiles of *Campylobacter* Species from infants presenting with diarrhea in Osun State, Nigeria. Open Journal of Medical Microbiology, 2015; 5 (1): 17–21.
doi: 10.4236/ojmm.2015.51003

[46] Ogbor O, Ajayi A, Zautner AE, Smith SI. Antibiotic susceptibility profiles of *Campylobacter coli* isolated from poultry farms in Lagos Nigeria– a pilot study. European Journal of Microbiology and Immunology, 2019; 9(2): 32-4. **doi: 10.1556/1886.2019.00007**

[47] Taylor DE, Courvalin P. Mechanisms of antibiotic resistance in *Campylobacter* species. *Antimicrobial Agents and Chemotherapy*, 1988: 32(8): 1107-12.

[48] Wieczorek K, Osek J. Antimicrobial resistance mechanisms among Campylobacter. Biomed Research International, 2013; 340605.
doi: 10.1155/2013/340605

[49] Zhao S, Young SR, Tong E, Abbott JW, Womack N, Friedman SL, et al. Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. Applied and Environmental Microbiology, 2010; 76 (24): 7949-56. **doi: 10.1128/AEM.01297-10**

[50] Wang Y, Taylor DE. Chloramphenicol resistance in *Campylobacter coli*: Nucleotide sequence, expression, and cloning vector construction. Gene, 1990; 94(1): 23–28.
doi: 10.1016/0378-1119(90)90463-2

[51] Jamali H, Ghaderpour A, Radmehr B, Wei KSC, Ching CL, Ismail S. Prevalence and antimicrobial resistance of *Campylobacter* species isolates in ducks and geese. Food Control, 2015; 50:328-30. **doi: 10.1016/j.foodcont.2014.09.016**

[52] Zendehbad B, Arian AA, Alipour A. Identification and antimicrobial resistance of *Campylobacter* species isolated from poultry meat in Khorasan province, Iran. Food Control, 2013; 32:724-7.
doi: 10.1016/j.foodcont.2013.01.035

[53] Fani F, Aminshahidi M, Firoozian N, Rafaatpour N. Prevalence, antimicrobial resistance, and virulence-associated genes of *Campylobacter* isolates from raw chicken meat in Shiraz, Iran. Iranian Journal of Veterinary Research, 2019; 20(4): 283-8.

[54] Martinez ADL, Lin J. Effect of an efflux pump inhibitor on the function of the multidrug efflux pump CmeABC and antimicrobial resistance in *Campylobacter*. Foodborne Pathogen Disease, 2006; 3: 393-402. **doi:10.1089/fpd.2006.3.393**