ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2019;5(1):49-60

Estimating the Frequency of *Campylobacter* spp. in Fecal Samples from Poultry Slaughterhouse Workers and Chicken Meat Sellers in Arak city, Iran

ARTICLE INFO

Article Type
Original Research

Authors
Ghorbani Marghmaleki E. *MSc*, Akbari M. * *PhD*, Arjomandzadegan M. *PhD*, Ahmadi A. *PhD*

How to cite this article
Ghorbani Marghmaleki E, Akbari M, Arjomandzadegan M, Ahmadi A. Estimating Frequency of *Campylobacter* spp. in Fecal Samples from Poultry Slaughterhouse Workers and Chicken Meats Sellers in Arak City, Iran. Infection Epidemiology and Microbiology. 2019;5(1):49-58

Microbiology Department, Infectious Diseases Research Center (IDRC), Arak University of Medical Sciences, Arak, Iran

* ***Correspondence***
Address: Department of Immunology and Microbiology, School of Medicine, Arak university of Medical Sciences, Arak, Iran
Phone: +98 9183613851-
Majakbari@yahoo.com

Article History
Received: February 26 ,2019
Accepted: May 1 ,2019
ePublished: May 30 ,2019

ABSTRACT

Aim: Thermophilic *Campylobacter* is the first cause of gastroenteritis infection in human. Nowadays, the prevalence of *Campylobacter* spp. is higher than other bacteria causing intestinal infection such as Salmonella and Shigella. This study was designed to compare the frequency of *Campylobacter* species in poultry slaughterhouse workers and poultry meat sellers (exposed group) and in healthy people (non-exposed group) in Arak city.

Materials & Methods: Among the 104 samples, 52 samples were collected from the slaughterhouse workers and poultry meat sellers, and 52 samples were collected from the control group. The stool samples were taken from the slaughterhouse workers, poultry meat seller, and healthy people who had not received antibiotics for the last two weeks. For enrichment, the samples were enriched in Preston broth medium at 37°C for 48 hrs under the microaerophilic conditions. Then they were sub cultured using a passive filtration method on Brucella agar at 37°C for 72 hrs under the microaerophilic conditions. Finally, the samples were directly tested using genus- and species specific PCR primers.

Findings: Of 52 samples collected from the slaughterhouse workers and poultry meat sellers, 11 (21.1%) samples were positive for the presence of *Campylobacter* spp. by PCR, and of 52 samples collected from the healthy people, 2 (3.8%) samples were reported as positive. The most frequent species isolated from the 2 groups were *C.jejuni* (53.84%) and *C.coli* (23.07%), respectively.

Conclusion: Chicken is identified as one of the important sources of *Campylobacter* infections in humans, which may contaminate poultry Slaughterhouse workers and chicken meat sellers, which in turn, they could potentially transmit *Campylobacter* strains to healthy people and chicken meat.

Keywords: Campylobacter, Slaughterhouse, Poultry products.

CITATION LINKS

[1] B1. Burnham PM, Hendrixson DR. Campylobac-ter jejuni: collective components promoting a successful enteric ... [2] Bronnec V, Turoňová H, Bouju A, Cruveiller S, Rodrigues R, Demnerova K, et al. Adhesion, biofilm formation, and genomic features of Campylobacter jejuni Bf, an atypical strain able to grow under aerobic .... [3] Johnson DI, Beck... [4] Chlebicz A, Śliżewska ... [5] Sakaridis I, Ellis RJ, Cawthraw... [6] Johnson TJ, Shank JM, ... [7] Dunn SJ, Pascoe B, Turton J, Fleming ... [8] Nyati KK, Nyati R. Role ... [9] Di Giannatale E, Di Serafino... [10] Facciolà A, Riso R, Avventuroso E, ... [11] Vinueza-Burgos C, Wautier M, Martiny D, ... [12] Corry J, Atabay H... [13] Althaus D, Zweifel ... [14] Linton D, Lawson A, Owen R, S... [15] Yamazaki-Matsune W, Taguchi M,... [16] Kawasaki S, Fratamico PM, Wesley IV, Kawamoto... [17] Torkan S, Vazirian B, Khamesipour... [18] Nilsson A, Skarp A, Johansson C, Kaden R, Engstrand L, ... [19] Tresse O, Alvarez-Ordóñez A, ... [20] Smith JL, Gunther IV NW. ... [21] Boysen L, Rosenquist H, Larsson J, ... [22] de Perio MA, Niemeier RT, Levine SJ, ... [23] Ellström P, Hansson I, Söderström C, ... [24] Porte L, Varela C, Haecker T, Morales S, Weitzel... [25] Holmberg M, Rosendal T, Engvall EO , Ohlson A, Lindberg A. Prevalence of thermophilic Campylobacter species in Swedish dogs and characterization of C. jejuni isolates. Acta veterinaria ... [26] Sainato R, ElGendy A, Poly F, Kuroiwa J, Guerry P, ... [27] Shams S, Bakhshi B, Nikmanesh B. Designing a rapid and accurate method for transportation and culture of the Campylobacter jejuni and Campylobacter coli-fastidious bacteria in the children with bacterial gastrointestinal ... [28] Ghosh R, Uppal B, Aggarwal P, Chakravarti A, Jha AK, Dubey A. A comparative study of conventional and molecular techniques in diagnosis of campylobacter gastroenteritis in children. Annals of ... [29] Wang H, Murdoch DR. Detection of Campylobacter species in faecal samples by direct Gram stain ... [30] Toledo Z, Simaluiza RJ, Astudillo X, Fernández H. Occurrence and antimicrobial susceptibility of thermophilic Campylobacter species isolated from healthy children attending .... [31] Shams S, Ghorbanalizadgan M, Haj Mahmmodi S, Piccirillo A. Evaluation of a Multiplex PCR Assay for the Identification of Campylobacter jejuni and Campylobacter coli. Infection, Epidemiology and Microbiology...

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

Introduction

Campylobacter spp. are considered as the important pathogens common among humans and domesticated animals worldwide [1]. *Campylobacter* species are typically gram-negative rods with spiral, curved, gull wing and seagull shapes. *Campylobacter* spp. are small (with 0.2–0.9 µm wide and 0.2 – 5.0 µm long) and doesn't have spores [2, 3]. They have a corkscrew-like movement [4]. *Campylobacter* is known to be a microaerophilic bacterium requiring to a specific conditions (10% CO2, 5% O2 and temperatures 37-42°C) for growth[5, 6].

After 24 to 48 hours of incubation, the colonies are gray, mucoid, and rough appearance, also similar to rain drop with metallic luminosity or no luminosity[7].

Among the *Campylobacter spp., C. jejuni, C. coli, C. hyointestinalis, C. upsaliensis, C. fetus*, *C. lari,* and *C. sputorum* are clinically of great importance. The most common clinical symptoms of *Campylobacter* infections include abdominal pain, diarrhea, headache and lethargy [8] Also about 10% of *Campylobacter* infections lead to the Guillain-Barre syndrome, Miller Fisher syndrome, Respiratory arthritis, Recurrent syndrome, Septicemia, and irritable bowel syndrome (IBS) [8, 9]. *Campylobacter* is transmitted to humans through contaminated foods, particularly chicken carcasses, vegetables, fish, milk and non-pasteurized dairy products [10].

Thermophilic *Campylobacter* is the first cause of gastroenteritis infection in humans [11]. These bacteria account for 2-10% of diarrheas in human, and the frequency of *Campylobacter* in slaughterhouses poultry meat was reported to be about 80% [12]. In slaughterhouses, even though the health principles are strictly observed, there are many opportunities for the transmission and spread of *Campylobacter* strains from poultry carcasses to slaughterhouse workers and healthy chickens [13].

Campylobacter spp. are fastidious bacteria due to the need for specific environmental conditions, specific medium, and so on for growth. As a result, no accurate information is available on the frequency of this bacterium in people at risk such as slaughterhouse workers, poultry meat seller, and healthy people.

Objective: Therefore, the purpose of this study was to compare the frequency of *Campylobacter* species in slaughterhouse workers and poultry meat sellers and in healthy people (control group).

Materials and Methods

Primary isolation: Code of medical ethics of this study was IR.ARAKMU. REC.1396.247. This study was a descriptive cross-sectional study conducted by easy sampling method during May 2018 to September 2018. Among the 104 samples, 52 cases were collected from the exposed group, including chicken slaughterhouse workers and chicken meat sellers and 52 samples were collected from the non-exposed group, including people whose jobs were not related to the chicken meat. The stool samples were collected from clients referred to the health centers of Arak city for obtaining health cards.

Inclusion criteria were as follows: not receiving antibiotics for at least two weeks before sampling and not having any clinical symptoms.

Gram staining of direct smear: First, using a sterile swab, fresh smear samples were prepared (sampling was done from muciod parts of samples having mucoid form). The smears were fixed using methanol. Modified gram staining was

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

performed for smears using a commercial Gram Staining Kit (Labtron, Iran). The opposite color was 3% fuchsin.

Culture: Fresh samples were inoculated in Periston broth (Ibresco.Iran.i23124) containing amphotericin B (10 mg / l), cycloheximid (10 mg / l), cefoperazone (8 mg / l), vancomycin (10 mg / l), rifampin (10 mg / l) and polymyxin (10 mg / l). Cultures were incubated at 37℃ in jar under the microaerophilic conditions (5% O2, 5% CO2, 10% H2 and 80% N2) for 72 hr. The microaerophilic conditions in the jar was provided using jar Whitley Gassing System.

Enriched samples were inoculated using passive filtration by 0.45 µm cellulose acetate filter (filter bio.china) on Brucella agar (Ibresco.Iran.i23026) containing 5% sheep blood. Brucella agar was incubated at 37℃ for 48 hrs under the abovementioned conditions. After 24 to 48 hours of incubation, the colonies are gray, mucoid, and rough appearance, also similar to rain drop with metallic luminosity or no luminosity. *Campylobacter* bacteria were obtained from the chicken specimens as the quality control and positive control of culture media.

DNA extraction from the stool: DNA extraction kit (Farogen, Iran) was used to purify DNA from stool. The core of the kit used in this study was columnar. The quality of the extracted DNA was confirmed using a nanodrop machine (Eppendorf, Germany), DNA samples were stored at -20℃.

PCR of the *16S rRNA* Gene: The PCR assay was designed to detect *Campylobacter* strains in genus level by *16S rRNA* gene with a product size of 856 bp [14]. The reaction mixture contained 7.5 µl Mastermix (Yekta Tajhiz Azma, Iran), 3.5 µl of DNA 50 ng and 0.7 µl of each forward and reverse primers (10 pmol)(Copenhagen, Denmark. The final volume was adjusted to 15 µl by adding distilled water in molecular grade. The PCR reaction was carried out in a thermocycler machine (Eppendorf, Germany) under the following temperature conditions: an initial denaturation step at 95℃ for 5 min, followed by 35 repeating cycles including denaturation at 95℃ for 1 min, annealing 56.4℃ for 55 sec, extension at 72℃ for 1 min, and a final extension at 72℃ for 10 min. PCR product was tested using 1% Agarose gel (Gene Fanavaran, Iran) electrophoresis (Padideh Nozhen Pars, Iran). Also, 1% agarose gel was studied by gel doc system (Quantum ST4. Germany). DNA extracted from *Campylobacter* colonies isolated from chicken was used as a positive control, and distilled water was used as a negative control.

Multiplex-PCR assay: Multiplex-PCR technique was used to identify *Campylobacter* species. The purpose of the multiplex-PCR technique was to detect the *C. coli*, *C.jejuni*, *C.fetus*, *C.hyointestinalis*, *C.lari* and *C.upsaliensis* species. The list of species-specific primers properties and the expected amplicon sizes are shown in Table 1. The reaction mixture contained 12.5 µl of PCR Mastermix (Yekta Tajhiz Azma, Iran), 3.5 µl of DNA (50 ng), and 0.7 µl of each primer) (Copenhagen, Denmark), and the final volume was adjusted to 25 µl by adding distilled water in molecular grade.

PCR conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 30 repeating cycles, including denaturation at 95 °C for 1 min, annealing at 56.4 °C for 55 sec, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR product was studied by gel doc system (Quantum ST4. Germany). DNA extracted from *Campylobacter* colonies isolated from chicken was used

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

Table1) Primers used to detect *Campylobacter* genus and species

Species	Target gene	Size (bp)	Primer	Sequence (5' to 3')	Reference
Campylobacter genus	*16 srRNA*	857	MD16S1 MD16S2	ATCTAATGGCTTAACCATTAAAC GGACGGTAACTAGTTTAGTATT	[14]
C. coli	*ASK*	502	CC18F CC519R	GGTATGATTTCTACAAAGCGAG ATAAAAGACTATCGTCGCGTG	[15]
C. jejuni	*Cj0414*	161	C-1 C-3	CAAATAAAGTTAGAGGTAGAATGT CCATAAGCACTAGCTAGCTGAT	[15]
C. fetus	*cst*	359	MG3F MG3F	GGTAGCCGCAGCTGCTAAGAT AGCCAGTAACGCATATTATAGTAG	[15]
C. hyointestinalis	*gyrB*	1250	CH F CH R	CGCCAAGAATTCGCCGAAGGCATACCTCAA CGCCAAGAATTCGCCGAAGGCATACCTCAA	[16]
C. lari	*glyA*	251	CL F CL R	TAGAGAGATAGCAAAAGAGA TACACATAATAATCCCACCC	[15]
C. upsaliensis	*lpxA*	86	CU61F CU146R	CGATGATGTGCAAATTGAAGC TTCTAGCCCCTTGCTTGATG	[15]

Table 2) Participants' demographic characteristics and the P-value of each attribute

Group	Character	Exposed		Non-exposed		P-value
		Frequency	Percentage	Frequency	Percentage	
Sex	Male	50	96.2 %	35	67.3 %	0.0001
	Female	2	3.8 %	17	32.7 %	
Address	Village	5	9.6 %	20	38.5 %	0.001
	City	47	90.4 %	32	61.5 %	
Marital status	Single	3	5.8 %	10	19.2 %	0.038
	Married	49	94.2 %	42	80.8 %	
Underlying illness	Yes	1	1.9 %	4	7.7 %	0.363
	No	51	98.1 %	48	92.3 %	
Symptoms	Yes	2	3.84 %	5	9.6 %	0.821
	No	50	96.16 %	47	90.4 %	
Tobacco use	Yes	47	90.4 %	16	30.8 %	0.0001
	No	5	9.6 %	36	69.2 %	
Age	20-35	39	75 %	41	78.8 %	0.598
	35-50	8	15.4 %	9	17.3 %	
	50-65	5	9.6 %	2	3.8 %	

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

as a positive control and distilled water, in molecular grade was used as a negative control. After data collection, data were analyzed using SPSS software and chi-squared test, and results were studied in exposed and non-exposed groups respectively

Findings

Direct smear: The direct smear of specimens were stained by modify gram staining method. According to the microscopic observations of the prepared slides, in 28 out of 52 samples from the exposed grou and in 11 out of 52 samples from the healthy people (non-exposed group), the result were positive for the presence of *Campylobacter*-like organisms (Fig. 1).

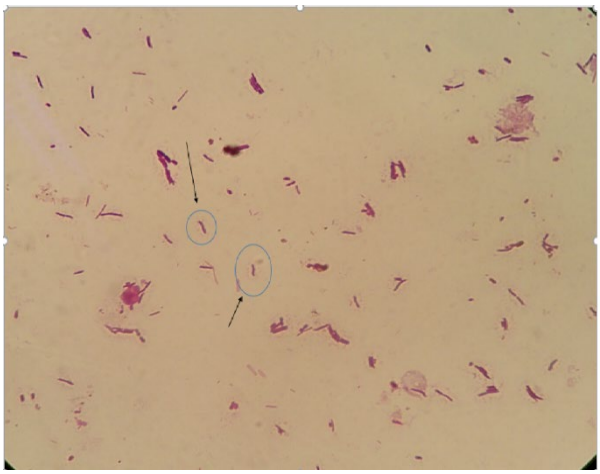

Figure 1) Gram stain modified by fuchsin color of stool specimen

Culture: Culture method results were negative for all human stool samples, and no colony was grown up on Brucella agar medium.

PCR of *16S rRNA* genes for identifying the genus *Campylobacter* from direct stool specimen: According to the PCR of *16S rRNA* gene for identifying the genus *Campylobacter* from the direct fecal specimens, among the 52 samples in the exposed group, 11 samples were positive (8 samples from slaughterhouse workers and 3 samples from poultry meat sellers). And 2 out of 52 samples in the non-exposed group, were positive for the presence of *Campylobacter* spp. (fig. 2).

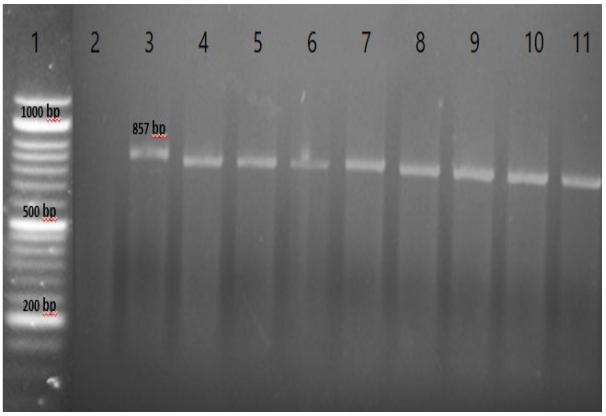

Figure 2) PCR results of *16S rRNA* gene to identify the genus *Campylobacter,* No. 1: Ladder 50bp, No. 2: Negative control, No. 3: Positive control, No.4 to No. 11: *Campylobacter* species

Sensitivity and specificity of direct smear in diagnosis of disease versus PCR:

Among the 104 smears, 28 slides (71.8%) of the exposed group and 11 slides (28.2%) of the non-exposed group were positive. Also, 11 out of 28 slides of the exposed groups, and 2 out of 11 slides of the non-exposed, were confirmed by PCR assay. PCR assay was considered as Gold standard. The remaining 26 samples were considered as samples containing *Campylobacter*-like organisms. Sensitivity and specificity of direct smear were calculated as, 76.92% and 13.63% respectively, using Medcalc software (Table 3). Also Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of direct smear were calculated as, 25.64% and 95.38% respectively, using Medcalc software (Table 3).

Multiplex-PCR for identifying *Campylobacter* species:

The results of Campylobacter species frequency in exposed and non-exposed groups and multiplex-PCR were presented in Table 4 and Fig. 3 , respectively.

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

Table 3) Sensitivity and specificity of direct smear

	Percentage	Confidence interval 95%	
		Up	Down
Sensitivity	76.92	46.19	94.96
Specificity	68.13	57.53	77.51
Positive likelihood ratio	2.41	1.58	3.68
Negative likelihood ratio	0.34	0.12	0.92
Positive predictive value	25.64	13.04	42.13
Negative predictive value	95.38	87.10	99.04

Table 4) Distribution of *Campylobacter* species in both case and control groups

Species	Group				P-Value
	Exposed		Non-exposed		
	Percentage	Frequency	Percentage	Frequency	
C. jejuni	9.6	5	3.8	2	0.04
C. coli	5.8	3	0	0	0.04
C. jejuni, C. hyointestinalis	3.8	2	0	0	0.04
C. upsaliensis	1.9	1	0	0	0.04
Negative	78.8	41	94.2	50	0.04

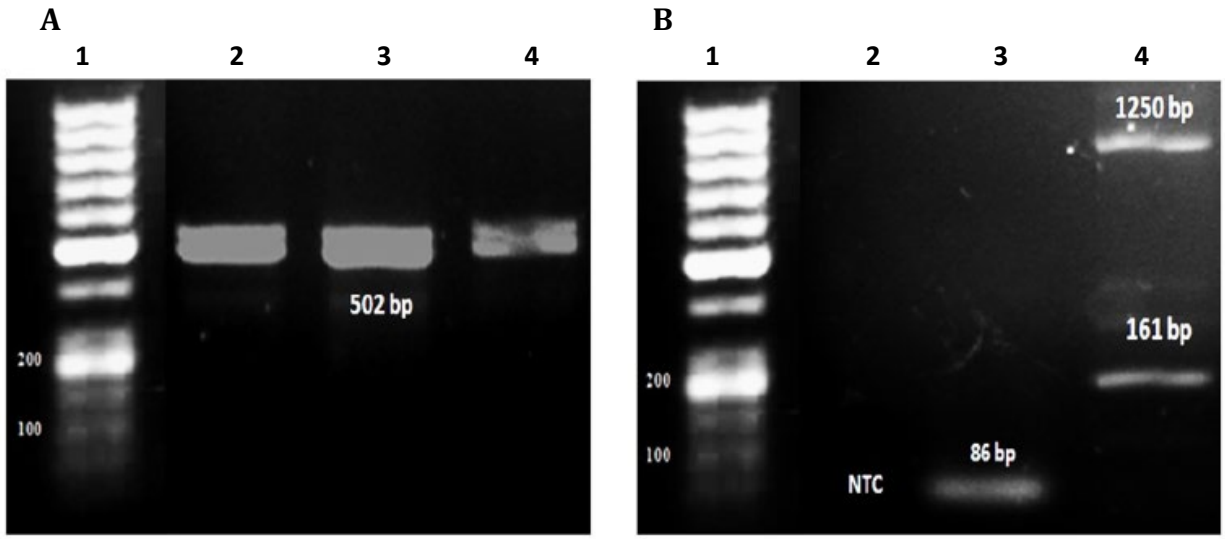

Figure 3) Multiplex-PCR results on electrophoresis gel. A) No.1: Ladder 50 bp, No.2: Positive control *C. coli* (502bp), No. 3, 4: *C. coli* (502bp). B) No.1: Ladder 50 bp, No.2: Negative control, No. 3: *C. upsaliansis* (86 bp), No. 4: *C. hyointestinalis* (1250 bp), and *C. jejuni* (161 bp).

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

Discussion

C. jejuni and *C.coli* are the most common *Campylobacter* species associated with gastroenteritis infection in humans, which are present in chicken bowel [17-19]. Due to the use of high temperatures and antibiotics in culture media, there is no precise information about the incidence of other non-thermophilic species [20, 21]. The incidence of *Campylobacter* infection around the world has been reported annually to be about 400 million people [14].

Most studies mainly consist of two groups of symptomatic patients as case and asymptomatic people as control group, in which asymptomatic group is divided into two groups of exposed and non-exposed to the contaminated material or equipment. In this study, as people under study were asymptomatic and healthy but at risk for the occupational exposure to *Campylobacter spp.,* and as the subject matter of this study has been rarely examined; therefore, to reach an accurate conclusion, this study results were, compared with the control groups, results of other studies.

In this study, among the 52 participants in the exposed group (slaughterhouse workers and poultry meat sellers), 50 (96.2%) cases were man and 2 (3.8%) cases were woman, and among the 52 participants in non-exposed group (healthy people), 35 (67.3%) cases were man and 17 (32.7%) cases were woman. Significant differences (p-value = 0.0001) were reported in the present study.

In a study by Marie et al. (2008-2011) in Virginia on slaughterhouse workers, among the 29 slaughterhouse workers, 28 (96.55%) cases were man, and the ratio of men in slaughterhouse workers was higher than women, [22] in their study, the ratio of men to women is consistent with the present study. In another study by Ellstrom et al. (2014) in Sweden, the proportion of women was reported to be higher than men, in which 17 cases were woman and 11 cases were man; in this regard, their study is not consistent with the present study [23]. According to their study, the reasons for the fewer number of women than men among the poultry slaughterhouse workers were inappropriate working hours and the remoteness of the slaughterhouse.

According to the results obtained in this study (Table 2), the frequency of smoking was higher in exposed individuals who were *Campylobacter* positive, which is likely due to the simultaneous tobacco smoking in working time. As a result, smoking can be considered as a risk factor for the *Campylobacter* infection.

Various studies have shown that gram staining of direct smear is one of the primary ways of detecting these bacteria in stool samples, which usually should be changed into the gram dyeing method in order to better view these bacteria, including the use of 3% fuchsine for 1 to 2 min. These bacteria are in the form of gram negative, weakly colored (sometimes shadow-like), spiral and, gull wings, which are also the properties of all *Campylobacter*-like organisms. In this study, Among the 104 smears, 28 slides (71.8%) in the exposed group and 11 slides (28.2%) in the non-exposed group were positive. Also 11 out of 28 slides of the exposed group, and 2 out of 11 slides of the non-exposed group were confirmed by PCR assay. The remaining 27 samples were considered as samples containing *Campylobacter*-like organisms. According to the direct smear results, the risk of infection with *Campylobacter* species in the exposed group was 2.545 times higher than in the non-exposed group. Direct microscopic observation approach depends on the expert's experience [24].

As there is no a similar study to the present study using direct smear for asymptomatic individuals, the interpretation of the present study results with the other studies results

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

seems to be illogical, indicating the need for further studies to be done in this area about the asymptomatic individuals because these people can be considered as healthy carriers in the community.

In this study, among the 104 samples collected from the slaughterhouse workers, poultry meat sellers, and healthy people, no colony was formed on Brucella agar medium. Ellstrom et al. also conducted a study in Sweden for two consecutive years to determine whether *Campylobacter* contamination could be transferred from poultry to slaughterhouse workers. In their study using culture method, only stool specimens of workers who were exposed to chicken meat for a long time were positive. It can be concluded that long-term exposure to chicken meat could be considered as a major problem for healthy people [23]. In this study, the low prevalence of *Campylobacter* species and the absence of clinical signs associated with *Campylobacter* infection in people under study may be attributed to the short duration of workers' employment in slaughterhouse, and consequently, the short duration of exposure to chicken meat, which can also be the reasons for lack of growth of *Campylobacter* colony on a specific medium. Most of the previous studies have been carried out on diarrheic patients with clinical symptoms, including fever, diarrhea, leukemia, and nausea [22], and on animals such as poultry, dogs, cats, and etc [17, 25]. As the *Campylobacter* infection dose was reported to be 9×10^4 [24, 26], and as the participants in this study were healthy and asymptomatic individuals; therefore, it is probably that the number of bacteria in these individuals has not reached the infection dose and not enough to cause symptoms and colonies.

Frequency of *Campylobacter* species by PCR: In this study, using PCR technique, among the 52 samples collected from the exposed group, 11 samples (22%) and among the 52 samples collected from the non-exposed group, 2 samples (4%) were positive; therefore; according to the PCR results, the risk of *Campylobacter* infection in the exposed group was 5.5 times higher than the other group, and p-value (0.008) was statistically significant.

In Ellstrom et al.'s (2014) study in Sweden, out of 28 health poultry slaughterhouse workers, 7 [22%] cases were positive by PCR assay, consistent with the present study results [23]. Marie et al. 's (20118-2011) study in Virginia on slaughterhouse workers shown that among the 1,000 slaughterhouse workers, 29 [2.9%) cases were positive using PCR, inconsistent with the present study results [22]

In other countries, poultry slaughterhouses use automated systems for chicken meat processing. Chicken drainage is also done by automatic machines; thus, in this way, all the chicken internal components are evacuated. But in Iran, manual handling is used for poultry processing and evacuation, and some parts of chicken visceral are remained. As a result, the higher frequency of *Campylobacter* spp. in Iran than other countries can be justified.

The results indicated that PCR assay was a very sensitive and useful method for the isolation of *Campylobacter* spp. It seems that the use of transport media such as Preston broth and *Campylobacter* optimization conditions can eliminate this bacterium colonies. In addition, Bita Bakhshi et al. (2016) in Iran designed a rapid and accurate method for the transportation and culture of the *C. jejuni* and *C. coli*-fastidious bacteria in children with bacterial gastrointestinal symptoms, and according to their study, PCR assay is a useful and sensitive method for the *Campylobacter* DNA extraction from feces and colonies [27].

Sensitivity and specificity of direct smear: Among the 104 smears, 28 slides (71.8%) in

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

the exposed group and 11 slides (28.2%) in the non-exposed group were positive. Also, 11 out of 28 slides in the exposed group, and 2 out of 11 slides in the non-exposed group were confirmed by PCR assay. The remaining 26 samples were considered as samples containing *Campylobacter*-like organisms. Sensitivity and specificity of direct smear were calculated as 76.92% and 13.63%, respectively, by Medcalc software. Negative predictive value (NPV) and positive predictive value (PPV) of direct smear were also calculated as 95.38% and 64.25%, respectively. In a study by Ghosh et al. (2014) in India, direct microscopy, culture, and PCR methods were compared to each other for the detection of *Campylobacter* spp. The sensitivity of direct microscopy, culture, and PCR methods was reported as 63.64%, 37.16%, and 96.66%, respectively. The direct smear method was reported to be better than the culture method in the selective medium [28]. In New Zealand, in 2004, the sensitivity and specificity of the direct gram staining method were reported to be 89% and 99.7%, respectively, for the detection of *Campylobacter* species in stool samples [29].

This method can be used to detect *Campylobacter* spp. due to its relatively high sensitivity and specificity in both patients and apparently healthy people. Direct gram staining method depends on the staining method and the expert's experience and thus requires standardization

Multiplex-PCR for species detection: Multiplex-PCR assay showed that *Campylobacter* spp. can be detected using 857bp target product of *16S rRNA* gene. According to the multiplex-PCR results, the most frequent isolated species in the exposed group was *C. jejuni* with a frequency of 40% (5 of 13), followed by *C. coli* with a frequency of 24% (3 of 13), *C. upsaliensis* with a frequency of 8% (1 of 13), and a combination of 2 *C. jejuni- C. hyointestinalis* species at the same time with a frequency of 16% (2 of 13). *C. jejuni* with a frequency of 16% (2 of 13) was the only species isolated from the non-exposed group.

In a study by Toledo et al. (2017) in South America on the prevalence of *Campylobacter* species in healthy children, the frequency of *C. jejuni* species was 52.94% (9 of 17), and the prevalence of *C. coli* was reported as 47.05% (8 of 17) [30]. Their study results were not matched with the present study results in the non-exposed groups.

In this study, *C. jejuni* was the most frequent isolated species with a frequency of 53.8% (7 of 13), this result was higher but consistent with the results of other studies conducted in Australia and Sweden on healthy individuals [23].

Shams et al. (2017) conducted a study in Iran to identify *C. jejuni* and *C. coli* species using multiplex-PCR assay. Among the 35 *Campylobacter* positive samples, 33 (94%) cases were identified as *C. Jejuni*, and 2 (6%) cases were identified as *C. coli*. These results showed that *C. jejuni* had the highest frequency among the *Campylobacter* spp. Also, in their study, the frequency of *C. jejuni* was higher than the *C. coli* species [31]. According to the previous studies, *C. coli* and *C.jejuni* are the most common *Campylobacter* species isolated from poultry; therefore, the result obtained in the present study are not unexpected.

Conclusion: This cross-sectional study showed that *Campylobacter* spp. as zoonotic agents, are asymptomatically able to clone in occupationally exposed human and possibly to cause these individuals to be *Campylobacter* carriers because this bacterium can be transmitted from these people to other people and to food products. It is recommended that more studies be done in order to understand the unknown dimensions of this bacterium epidemiology.

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

Acknowledgement: This article is based on the dissertation of Elahe Ghorbani, sponsored by the Arak Medical University. The authors would like to thank from all those involved in the implementation of this project, including Mrs. Talebi and Mrs. Aysan Karami.

Conflict of Interests: The authors declare they have no conflict of interests.

Ethical Permissions: The number of medical ethics of this study was IR.ARAKMU.REC.1396.247

Authors' Contribution: Elahe Ghorbani Marghmaleki (Introduction author, Original researcher and Discussion author); Majid Akbari and Mohammad Arjomandzadegan (Methodologist); Azam Ahmadi (Statistical analyst).

Funding: No competing financial interests exist.

References

1. Burnham PM, Hendrixson DR. Campylobacter jejuni: collective components promoting a successful enteric lifestyle. Nature Reviews Microbiology. 2018:1.
2. Bronnec V, Turoňová H, Bouju A, Cruveiller S, Rodrigues R, Demnerova K, et al. Adhesion, biofilm formation, and genomic features of Campylobacter jejuni Bf, an atypical strain able to grow under aerobic conditions. Frontiers in microbiology. 2016;7:1002.
3. Johnson DI, Beck. Bacterial Pathogens and Their Virulence Factors: Springer; 2018.
4. Chlebicz A, Śliżewska K. Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. International journal of environmental research and public health. 2018;15(5):863.
5. Sakaridis I, Ellis RJ, Cawthraw SA, Van Vliet AH, Stekel DJ, Penell J, et al. Investigating the association between the caecal microbiomes of broilers and Campylobacter burden. Frontiers in microbiology. 2018;9.
6. Johnson TJ, Shank JM, Johnson JG. Current and potential treatments for reducing Campylobacter colonization in animal hosts and disease in humans. Frontiers in microbiology. 2017;8:487.
7. Dunn SJ, Pascoe B, Turton J, Fleming V, Diggle M, Sheppard SK, et al. Genomic epidemiology of clinical Campylobacter spp. at a single health trust site. Microbial genomics. 2018;4(10).
8. Nyati KK, Nyati R. Role of Campylobacter jejuni infection in the pathogenesis of Guillain-Barré syndrome: an update. BioMed research international. 2013;2013.
9. Di Giannatale E, Di Serafino G, Zilli K, Alessiani A, Sacchini L, Garofolo G, et al. Characterization of antimicrobial resistance patterns and detection of virulence genes in Campylobacter isolates in Italy. Sensors. 2014;14(2):3308-22.
10. Facciolà A, Riso R, Avventuroso E, Visalli G, Delia SA, Laganà P. Campylobacter: From microbiology to prevention. Journal of preventive medicine and hygiene. 2017;58(2):E79.
11. Vinueza-Burgos C, Wautier M, Martiny D, Cisneros M, Van Damme I, De Zutter L. Prevalence, antimicrobial resistance and genetic diversity of Campylobacter coli and Campylobacter jejuni in Ecuadorian broilers at slaughter age. Poultry science. 2017;96(7):2366-74.
12. Corry J, Atabay H. Poultry as a source of Campylobacter and related organisms. Journal of Applied Microbiology. 2001;90(S6):96S-114S.
13. Althaus D, Zweifel C, Stephan R. Analysis of a poultry slaughter process: Influence of process stages on the microbiological contamination of broiler carcasses. Italian journal of food safety. 2017;6(4).
14. Linton D, Lawson A, Owen R, Stanley J. PCR detection, identification to species level, and fingerprinting of Campylobacter jejuni and Campylobacter coli direct from diarrheic

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

samples. Journal of clinical microbiology. 1997;35(10):2568-72.
15. Yamazaki-Matsune W, Taguchi M, Seto K, .Kawahara R, Kawatsu K, Kumeda Y, et al. Development of a multiplex PCR assay for identification of Campylobacter coli, Campylobacter fetus, Campylobacter hyointestinalis subsp. hyointestinalis, Campylobacter jejuni, Campylobacter lari and Campylobacter upsaliensis. Journal of Medical Microbiology. 2007;56(11):1467-73.
16. Kawasaki S, Fratamico PM, Wesley IV, Kawamoto S. Species-specific identification of campylobacters by PCR-restriction fragment length polymorphism and PCR targeting of the gyrase B gene. Applied and environmental microbiology. 2008;74(8):2529-33.
17. Torkan S, Vazirian B, Khamesipour F, Dida GO. Prevalence of thermotolerant Campylobacter species in dogs and cats in Iran. Veterinary medicine and science. 2018;4(4):296-303.
18. Nilsson A, Skarp A, Johansson C, Kaden R, Engstrand L, Rautelin H. Characterization of Swedish Campylobacter coli clade 2 and clade 3 water isolates. MicrobiologyOpen. 2018:e00583.
19. Tresse O, Alvarez-Ordóñez A, Connerton IF. About the Foodborne Pathogen Campylobacter. Frontiers in microbiology. 2017;8:1908.
20. Smith JL, Gunther IV NW. Commentary: Campylobacter and Hemolytic Uremic Syndrome. Foodborne pathogens and disease. 2018.
21. Boysen L, Rosenquist H, Larsson J, Nielsen E, Sørensen G, Nordentoft S, et al. Source attribution of human campylobacteriosis in Denmark. Epidemiology & Infection. 2014;142(8):1599-608.
22. de Perio MA, Niemeier RT, Levine SJ, Gruszynski K, Gibbins JD. Campylobacter infection in poultry-processing workers, Virginia, USA, 2008–2011. Emerging infectious diseases. 2013;19(2):286.
23. Ellström P, Hansson I, Söderström C, Engvall EO, Rautelin H. A prospective follow-up study on transmission of Campylobacter from poultry to abattoir workers. Foodborne pathogens and disease. 2014;11(9):684-8.
24. Porte L, Varela C, Haecker T, Morales S, Weitzel T. Impact of changing from staining to culture techniques on detection rates of Campylobacter spp. in routine stool samples in Chile. BMC infectious diseases. 2016;16(1):196.
25. Holmberg M, Rosendal T, Engvall EO, Ohlson A, Lindberg A. Prevalence of thermophilic Campylobacter species in Swedish dogs and characterization of C. jejuni isolates. Acta veterinaria scandinavica. 2015;57(1):19.
26. Sainato R, ElGendy A, Poly F, Kuroiwa J, Guerry P, Riddle MS, et al. Epidemiology of Campylobacter Infections among Children in Egypt. The American journal of tropical medicine and hygiene. 2018;98(2):581-5.
27. Shams S, Bakhshi B, Nikmanesh B. Designing a rapid and accurate method for transportation and culture of the Campylobacter jejuni and Campylobacter coli-fastidious bacteria in the children with bacterial gastrointestinal symptoms. Koomesh. 2016;18(1).
28. Ghosh R, Uppal B, Aggarwal P, Chakravarti A, Jha AK, Dubey A. A comparative study of conventional and molecular techniques in diagnosis of campylobacter gastroenteritis in children. Annals of Clinical & Laboratory Science. 2014;44(1):42-8.
29. Wang H, Murdoch DR. Detection of Campylobacter species in faecal samples by direct Gram stain microscopy. Pathology. 2004;36(4):343-4.
30. Toledo Z, Simaluiza RJ, Astudillo X, Fernández H. Occurrence and antimicrobial susceptibility of thermophilic Campylobacter species isolated from healthy children

[DOR: 20.1001.1.25884107.2019.5.1.7.8]

attending municipal care centers in Southern Ecuador. Revista do Instituto de Medicina Tropical de São Paulo. 2017;59.
31. Shams S, Ghorbanalizadgan M, Haj Mahmmodi S, Piccirillo A. Evaluation of a Multiplex PCR Assay for the Identification of Campylobacter jejuni and Campylobacter coli. Infection, Epidemiology and Microbiology. 2017;3(1):6-8.

[DOR: 20.1001.1.25884107.2019.5.1.7.8]