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Evaluating the Frequency of *Arcobacter* in Fecal Samples from Slaughterhouse Workers and Poultry Meat Sellers in Arak City

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ABSTRACT

Aims: In recent years, *Arcobacter* has been isolated from various samples. It can cause diseases both in human and animal and be transmitted to human through water, food, and continuous contact with poultry meat. Therefore, people exposed to the contaminated meat such as chicken meat can be exposed to *Arcobacter* too and as a part of its transmission route. Thus, in this study, the frequency of *Arcobacter* species was evaluated in slaughterhouse workers and poultry meat sellers and healthy people not exposed to the poultry meat.

Materials & Methods: In the present study, 85 slaughterhouse workers and poultry meat sellers (exposed group) and 85 healthy people with other jobs (non-exposed group) were studied. By simple method, fecal samples were collected from Health Center of Arak city and tested by 4 methods including direct observation, culture, PCR, and m-PCR.

Findings: *Campylobacter*-like organisms were observed in 32 out of 85 samples from the exposed group and in 11 out of 85 samples from the non-exposed group by microscopic observation method. No sample was positive by culture method. However, by PCR method, the frequency of *Arcobacter* strains was 20 in the exposed group and 6 in the non-exposed group. According to the m-PCR results, among the 170 samples, 21 *A. cryaerophilus* and 14 *A. butzleri* strains were identified.

Conclusion: Chicken carcass are introduced as a main reservoir for *Arcobacter*; therefore, continuous contact with poultry meat can have a significant effect on the transmission of *Arcobacter* strains to individuals. Therefore, this study showed that the frequency of *Arcobacter* strains is more in exposed group than in non-exposed group.

Keywords: *Arcobacter*; Slaughterhouse workers; Poultry meat sellers

CITATION LINKS

[1] its public health concerns and advances in... [2] Identification by 16S ribosomal RNA gene sequencing of... [3] isolated from an estuarine sediment and... [4] Efficacy of wastewater treatment on ... [5] Comparative detection and quantification of... [6] Arcobacter butzleri an emerging enteropathogen... [7] cryaerophilus in human... [8] Arcobacter spp. at retail food from... [9] Detection of Arcobacter butzleri and Arcobacter cryaerophilus in clinical samples of humans and foods of animal origin by cultural ... [10] Isolation of Group 2 aerotolerant Campylobacter species from Thai children with diarrhea... [11] Prevalence of Arcobacter species among humans, Belgium... [12] Rao UA. Prevalence of Campylobacter jejuni and enteric bacterial pathogens among hospitalized HIV infected versus non-HIV infected patients with diarrhoea in southern India... [13] detection and enumeration of Campylobacteraceae in irrigation water and wastewater using a miniaturized MPN-qPCR assay... [14] characterization of the emerging foodborn pathogen ... [15] Phenotypic and ribosomal RNA characterization of Arcobacter species isolated from porcine aborted fetuses... [16] Occurrence and diversity of Arcobacter... [17] Hausdorf L, Fröhling A, Schlüter O, Klocke M. Analysis of the bacterial community within carrot wash water. Can J Microbiol... [18] Milesi S. Emerging pathogen Arcobacter spp. in food of animal origin [19] Occurrence and genetic diversity of Arcobacter spp. in a spinach-processing plant and evaluation of two Arcobacter-specific quantitative PCR assays. .. [20]. isolated from humans and animals with diarrheal illness... [21] Occurrence of Arcobacter in Iranian poultry and slaughterhouse samples ... [22] Arcobacter butzleri, a new waterborne pathogen, to water distribution pipe surfaces... [23] detection methods for Arcobacter infections in diarrhea specimens among children under six years in ... [24] multiplex PCR assay for the simultaneous detection and identification of Arcobacter butzleri, Arcobacter cryaerophilus and Arcobacter skirrowii... [25] A selective medium for the isolation of Arcobacter from meats... [26] Research methods for the behavioral sciences: Cengage ... [27] Prevalence of Campylobacter, Arcobacter, Helicobacter... [28] Induction and resuscitation of viable nonculturable Arcobacter ... [29] Filling the gaps in clinical proteomics: a do-it-yourself guide for the identification of the emerging pathogen Arcobacter by... [30] Evaluation of detection methods for Campylobacter...

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Introduction

Arcobacter spp. are introduced as food and water borne pathogens which can cause various diseases in humans, including gastroenteritis, bacteremia, endocarditis, peritonitis, and diarrhea [1-2]. This organism is a Gram-negative, curved, spiral or bacilli-shaped, and non-spore forming bacterium which can move fast like a corkscrew due to its monotrichous or amphitrichous flagella. Its length and width are 1 to 3 µm and 0.2 to 0.9 µm, respectively [3]. *Arcobacter* is a new genus introduced in 1991 by Vandamme *et al*. The genus *Arcobacter* is closely related to the *Campylobacter* species and a member of family *Campylobacteracea*e [4]. *Arcobacter* can be differentiated from *Campylobacter* by growing in air at the temperatures of 15 to 30 °C [3]. Among its species, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* species are clinically of great importance [5]. The most common culture method for the detection of *Arcobacter* strains is samples enrichment with CAT broth and inoculation of enriched samples on agar media with 5% defibrinated sheep blood by passive filtration method (size of filters 0.45 micron) [6-7]. In several studies, molecular methods were used to detect *Arcobacter* spp. PCR and multiplex-PCR assays were mostly used for the detection of *Arcobacter* at genus and species levels, respectively [7-9]. The prevalence of *Arcobacter* spp. was reported to be 57% in adult with diarrhea and 2.4% in children with diarrhea [10]. whereas this bacterium is considered as the second cause of diarrhea after *Campylobacter spp*. in Iran, it is introduced as the fourth common species causing diarrhea in South Africa, Belgium, and France [11]. In a study conducted in India, it was isolated from 1.5% of fecal specimens of HIV positive patients with diarrhea [12]. In another study, *Arcobacter* spp. were isolated from stools of healthy people, especially those dealing with animal origin products and working in slaughterhouses [13]. In this regard, in a study conducted in Netherlands (2006), *A. cryaerophilus* species was found in 1.4 % of fecal samples of slaughterhouse workers, meat sellers, and healthy people not exposed to chicken meat [14]. *Arcobacter spp*. can cause diarrhea, mastitis, and abortion in animals, it can be also isolated from healthy animals [15]. Animals can act as a major reservoir and source of *Arcobacter* infection for humans. Animal-origin food products, contaminated vegetables, and dairy products are thought to be as the potential transmission routes for *Arcobacter* [16]. *Arcobacter spp.* have been isolated from various samples of chicken, pig, cow, and seafood products [17]. According to the research, contamination rate of poultry meat is higher than red meat; thus, *Arcobacter* infection may be occurred in humans due to direct contact and consumption of contaminated raw meat [18]. Furthermore, water plays a main role in the contamination of vegetables and slaughtered animals during washing [19]. Approximately, 63% of diseases caused by *A. butzleri* are associated with the consumption of or contact with contaminated water [20]. Several studies demonstrated the presence of *A. butzleri* and *A. cryaerophilus* species on the instruments used in slaughterhouses and chicken meat shops; therefore, cross-contamination may occur by contacting them [21]. The consecutive proliferation of *Arcobacter* in slaughterhouses and chicken meat shops environment is mainly due to the biofilm formation ability of *Arcobacter* strains on physical surfaces [22]. Therefore, it is thought that slaughterhouse workers and chicken meat sellers can be mainly exposed to *Arcobacter.* In other words, they can act as healthy carriers for *Arcobacter*.

Objective: This study aimed to investigate

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the frequency of *Arcobacter* strains in poultry slaughterhouse workers and poultry meat sellers.

Materials and Methods

Sample collection: This study was a descriptive study. Sample collection was carried out during May to September 2018. A total of 170 samples were collected at Health Center of Arak city, Iran and divided into two groups of exposed and non-exposed groups. About 85 fecal samples collected from chicken meat sellers and slaughterhouse workers were included in the exposed group, and 85 fecal samples collected from individuals with other jobs were included in the non-exposed group. According to the used questionnaire, people working at slaughterhouses or poultry meat shops and not taking antibiotics were listed in the exposed group, and those having no chicken-related job and not using any antibiotics were listed in the non-exposed group.

Gram staining: Bacterial smears from mucoid parts of stool were stained by modified Gram staining method, in which fuchsin 3% solution was used [23].

Isolation of *Arcobacter*: Fecal samples were enriched using Arco broth media containing cefoperazone, amphotericin B, and teicoplanin (CAT) antibiotics and incubated for 48 hr at 28 °C in candle jar. After that, enriched fecal samples were inoculated on Brucella agar medium containing 5% defibrinated sheep blood by passive filtration method using cellulose acetate filter membrane with pour size of 0.45 µm. Afterwards, the plates were incubated for 48 hr at 28 °C in candle jar [7].

Quality control of culture media: In order to control the quality of culture media, *Arcobacter* strains isolated from chicken specimens were used. They were cultivated according to the mentioned principles.

DNA extraction: DNA extraction from direct fecal samples was carried out by a commercial kit (FAVORGEN, Taiwan) through cellulose acetate columns.

Genus specific-PCR: *16SrRNA* gene was targeted to identify *Arcobacter* strains in genus level by PCR [23]. Arc1 (5'AGAACGGGTTATAGCTTGCTAT3') and Arc2 (5'GATACAATACAGGCTAATCTCT3') were used as primers. PCR mixture was prepared in a final volume of 15 µl containing 1.5 µl of DNA template, 7.5 µl of Master Mix Super-PCR (Yekta Tajhiz Azma Co.), 0.7 µl of each primers, and 4.6 µl of water with molecular grade. PCR was performed in thermal cycler (Eppendorf, Germany) under the following conditions: an initial denaturation at 94 °C for 5 min, followed by 28 cycles including denaturation step at 94 °C for one min, annealing step at 52.7 °C for 55 sec, extension step at 72 °C for 55 sec, and a final extension step at 72 °C for 8 min. The Genome obtained from *Arcobacter* colonies was used as positive control, and distilled water with molecular grade was utilized as negative control. The PCR product was checked using gel electrophoresis (padideh nojen, Iran).

Specific-PCR (m-PCR): This method was used to identify three important *Arcobacter* species [24]. Specific primers of each species are shown in Table 1. The PCR mixture was prepared in a final volume of 15 µl containing 3 µl of DNA template, 6.2 µl of Master Mix Super-PCR (Yekta Tajhiz Azma Co.), 0.7 µl of each primers, and 4.4 µl of distilled water with molecular grade. PCR was performed in thermal cycler (Eppendorf, Germany) under the following conditions: an initial denaturation at 94 °C for 5 min, followed by 35 cycles including denaturation step at 94 °C for one min, annealing step at 52.7 °C for 55 sec, extension step at 72 °C for 1 min, and final extension step at 72 °C for 10 min. PCR product was examined using gel electrophoresis (padideh nojen, Iran).

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Table 1) Characteristics of Specific primers used to determine Arcobacter species

Name of the primer	Sequence (5′ to 3′)	Target gene	Product size (bp)	annealing	references
Arc 1 (F) Arc 2 (R)	AGAACGGGTTATAGCTTGCTAT GATACAATACAGGCTAATCTCT	16SrRNA (genus)	181	52.7° C	(25)
Butz (F) Arco (R)	CCTGGACTTGACATAGTAAGAATGA CGTATTCACCGTAGCATAGC	16SrRNA (*butzleri*)	401	52.7° C	(30)
CryF (F) CryR (R)	CAGAGGAAGAGAAATCAAAT CCCACTATTCCATCAGTGAG	23SrRNA (*cryaerophilus*)	257	52.7° C	(30)
SkiR (R) ArcoF(F)	TCAGGATACCATTAAAGTTATTGATG GCYAGAGGAAGAGAAATCAA	23SrRNA (*skirrowii*)	198	52.7° C	(25)

Findings

Sample collection: Demographic information of both groups are shown in Table 2.

Table 2) Shows the frequency distribution of the characteristics of individual in each group based on the questionnaire

		Exposed		Non-exposed		p-value
		Frequency	Percentage	Frequency	Percentage	
Sex	Male	83	97.6	63	74.1	0.0001
	Female	2	2.4	22	25.9	
Life position	Village	8	9.4	27	31.8	0.0001
	City	77	90.6	58	68.2	
Personal status	Single	30	35.3	14	16.4	0.005
	Married	55	64.7	71	83.6	
Cigarette use	Yes	62	72.9	37	43.5	0.001
	No	23	27.1	48	56.5	
Symptoms	Yes	8	9.4	12	14.1	0.341
	No	77	90.6	73	85.9	
Underlying diseases	Yes	3	3.5	9	10.6	0.072
	No	82	96.5	76	89.4	
Stool appearance	Diarrhea	6	7.1	4	4.7	0.514
	Non-diarrhea	79	92.9	81	95.3	
Age	20-35	43	50.6	56	65.9	0.126
	36-50	30	35.3	20	23.5	

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Gram staining: According to the microscopic observations, of 85 fecal samples collected from the exposed group, 32 (37.64%) cases were positive for *Campylobacter*-like organisms, and among the 85 fecal samples collected from the non-exposed group, 11 (12.94%) cases were reported as positive (Figure 1).

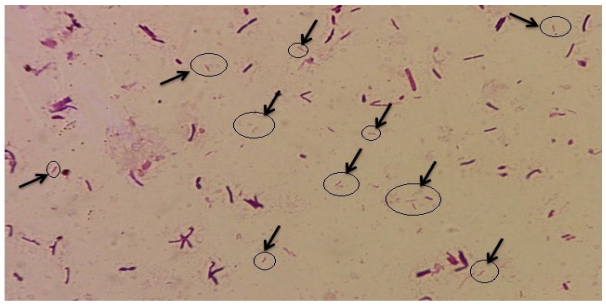

Figure 1) Gram stained bacterial smear from stool samples indicates Campylobacter like-organisms (curved, bacilli and gram negative organisms)

Culture: In all samples, no colony was grown.
Genus-specific PCR: PCR results showed that of 85 fecal samples collected from the exposed group, 20 (23.5%) samples were positive, and of 85 samples collected from the non-exposed group, 6 (7.5%) samples were positive (Figure 2).

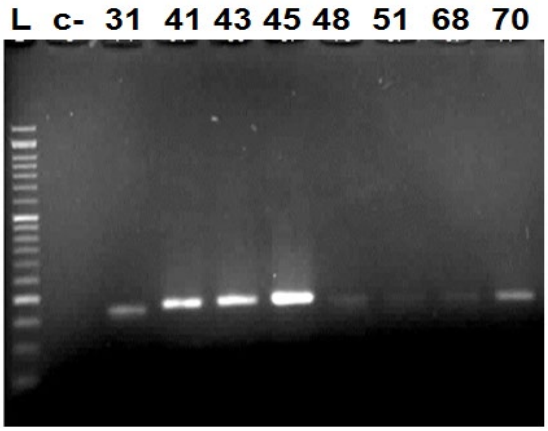

Figure 2) Agarose gel electrophoresis of Genus specific-PCR product (181bp): 31,41,43,45,51 lanes belong to slaughterhouse workers' samples, 48.68.70 lanes are samples of unexposed group and 68.70 lanes are related to chicken meat sellers' samples and C- lane is as negative controls. The Ladder size is 50bp (Yekta Tajhiz Azma Co.).

Species-specific PCR: Species-specific PCR indicated that in the exposed group, *A. cryaerophilus* species was detected in 9 (10.58%) samples, *A. butzleri* species was detected in 4 (4.70%) samples, and both *A. cryaerophilus* and *A. butzleri* species were detected in 7 (8.23%) samples. In the non-exposed group, *A. cryaerophilus* species was detected in 3 (3.52%) samples, *A. butzleri* species was detected in 1 (1.17%) sample, and both species were detected in 2 (2.35%) specimens. No *A. skirrowii* species was detected in fecal samples (Figure 3).

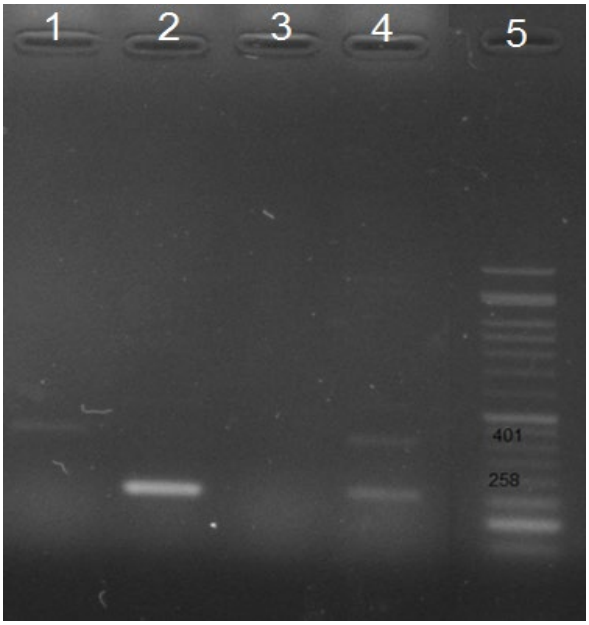

Figure3) the results of m-PCR on agarose gel electrophoresis. Lanes: 1: A.butzleri (401bp), 2: A. cryaerophilus (258bp), 3: Negative control, 4:both, 5: Ladder with size of 50bp (Yekta Tajhiz Azma Co.).

Statistical analyses: All data were statistically significant ($p<0.005$).

Discussion

Arcobacter is introduced as a common pathogen among humans and animals. Over the past decade, due to the early reports of *Arcobacter* strains isolation from animal-origin foods, *Arcobacter* has become more important for human public health, because of causing diarrhea and bacteremia in

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humans and animals. In addition to diarrhea and bacteremia, it also causes diseases in animals. However, some studies conducted worldwide reported the presence of *Arcobacter* species in raw meat, especially poultry meat, and emphasized that continuous contact with contaminated meat may be a potential transmission route of *Arcobacter* to humans [25].

It is assumed that poultry slaughterhouse workers or chicken meat sellers who are exposed to poultry carcasses can be as the potential carriers for *Arcobacter*. Therefore, this study aimed to detect *Arcobacter* species in fecal specimens of people exposed to and non-exposed to the chicken meat. In the present study, the frequency distribution of smoking in two groups was statistically significant, and the ratio of smoking frequency was higher in the exposed group. Based on the direct microscopic observation of smears from symptomatic people in different studies, modified Gram straining method is considered as one of the main methods to identify *Arcobacter* species, in which the use of 3% fuchsin instead of normal fuchsin for 1-2 min is usually suggested. It can improve the *Arcobacter* strains observation because they can be observed as Gram negative with a weak tonality (sometimes like-shadow) and spiral, making them to be reported as *Campylobacter*-like organisms. Sensitivity and specificity of this method are very high. In the present study, according to the direct observations results,, *Campylobacter*-like organisms were observed in 32 (37.64%) smears belonging to exposed group, whereas they were observed in 11 (12.94%) smears belonging to non-exposed group. Data were statistically significant in both groups, and the frequency of the positive results was higher in the exposed group.

In a study by Khalili *et al*. (2018) in Iran, from among the 150 fecal samples collected from people with diarrhea, *Campylobacter*-like organisms were observed in 79 smears [23]. This technique depends on laboratory expert's experience, which can be the reason for variability in various studies results [26]. In other studies, the studied population mainly included asymptomatic healthy people and patients named symptomatic people; however, asymptomatic individuals were divided into two groups of exposed and non-exposed to bacteria, they were participated to be used as criteria to compare the results of groups. But the present study focused on asymptomatic people divided into exposed and non-exposed groups, in which the poultry slaughterhouses workers and chicken meat sellers were included in exposed group. In the present study, no *Arcobacter* species was isolated using culture method. In a study by Houf *et al.* (2006) in Switzerland, from a total of 501 fecal specimens collected from asymptomatic people, *Arcobacter* strains were isolated only from 7 (1.4%) samples using enriched culture [14]. In another study by Enberg *et al*. in Denmark (2000), no *Arcobacter* strain was isolated from 107 healthy individuals by culture method [27]. The frequency of culture positive results of asymptomatic healthy people in other studies was also very low, and in some studies, it even reached nearby zero; *Arcobacter* susceptibility to antimicrobial agents existing in these culture media is probably one of its reasons. Fera *et al*. (2008) suggested that the low level of *Arcobacter* detection in fecal specimens using enriched culture media may be due to the competition of normal flora with *Arcobacter* strains [28]. Other studies also similarly confirmed the defective specificity of culture media to isolate *A. butzleri* isolates from stool specimens. According to the genus-specific PCR results, of 26 positive cases, 20 (76.9%) positive samples belonged to the exposed group, and 6 (23.1%) positive

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samples belonged to the non-exposed group. Data were statistically significant, and the frequency of positive results in the exposed group was higher. In a study by Webb *et al*. (2016) in Canada, using PCR, the prevalence of *Arcobacter* strains was reported as 60% among the 1482 patients and 88 asymptomatic healthy people. In their study, *Arcobacter* spp. were found in 57% and 46% of patients and asymptomatic healthy people, respectively, confirming the present study results [5]. In the present study, *Campylobacter*-like organisms were observed in 43 direct Gram-stained smears. However, the presence of *Arcobacter* strains was confirmed just in 13 smears using PCR. If PCR is considered as a gold standard, the sensitivity and specificity of the Gram staining method for detecting *Arcobacter* species in asymptomatic individuals were 50% and 71.17%, respectively. In a study by Khalili *et al.* (2017) in Iran, among the 79 Gram-stained smears from symptomatic individuals who were positive for *Campylobacter*-like organisms, 28 smears were identified as *Arcobacter* using PCR. In their study, the sensitivity and specificity of the Gram staining method for detecting *Arcobacter* spp. were 100% and 65.5%, respectively [...]. However, it should be noted that all positive smears are not always related to the *Arcobacter* species, since Gram-negative, curved, and spiral-shaped bacteria in smears are reported as *Campylobacter*-like organisms, including *Campylobacter*, *Arcobacter*, and *Helicobacter* spp. [23]. According to the multiplex-PCR results, from among 170 samples, 21 (12.35%) *A. cryaerophilus* and 14 (8.22%) *A. butzleri* strains were identified. Among the exposed group, *A. cryaerophilus* species was identified in 9 samples, *A. butzleri* species was identified in 4 samples, and both species were identified in 7 samples, while in the non-exposed group, *A. cryaerophilus* species was identified in 3 samples, *A. butzleri* species was identified in 1 sample, and both species were identified in 2 samples. No *A. skirrowi* species was detected in samples. In a study *by* Houf *et al*. (2006), *Arcobacter* strains were detected in 7 out of 501 healthy individuals' stool samples, all of which were identified as *A. cryaerophilus* [14]. In another study by Khalili *et al.* (2018) in Iran, from a total of 230 diarrheal samples collected from symptomatic individuals, 26 (11.30%) cases were identified as *A. butzleri*, 13 cases as *A. cryaerophilus,* and 5 cases as *A. skirrowii* [23]. *Arcobacter* different detection levels reported in different studies can be associated with different factors, including geographical variations, seasonal variations, health conditions in different locations, the number of microorganisms in samples under study, and difference in sensitivity and specificity of isolation methods used [29]. Despite the lack of colony growth on the media and limitations in access to healthy exposed people during sample collection, DNA-based methods were more suitable to detect *Arcobacter* and its distinct species.

Conclusion

Poultry raw meat can be a very important reservoir for the transmission of *Arcobacter* to humans. Consequently, workers' and Sellers' direct contact with poultry raw meat in slaughterhouses and shops and smoking during work could have a significant effect on the transmission of this bacterium to them and probably on making them as the *Arcobacter* carriers. Therefore, poultry slaughterhouse workers and chicken meat sellers can be healthy while potentially *Arcobacter* carriers. Accordingly, there is a possibility of these bacteria transmission from these individuals to healthy poultry meat or to healthy individuals. In addition, identification and treatment of asymptomatic individuals exposed to the risk of

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contamination can reduce this bacterium prevalence in population. It is suggested that direct observation method be used as a very effective method to diagnose *Arcobacter* strains in healthy people, and PCR as a method making *Arcobacter* detection fast.

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