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Prevalence, Comparison of Diagnostic Methods, Antibiogram, and Genotyping of *Arcobacter* spp. in Diarrheal Cases Referring to Clinical Centers in Iran

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ABSTRACT

Backgrounds: *Arcobacter* is an emerging bacterium that may cause watery diarrhea and septicemia in humans. This study aimed to investigate the prevalence of *Arcobacter* spp. in diarrheal stool specimens using culture and molecular methods, their genetic diversity, and their resistance to different antibiotics in patients referring to clinical centers in Arak, Iran.
Materials & Methods: In this descriptive cross-sectional study, diarrheal stool specimens were collected from 230 patients over a two-month period from July to September 2016. The samples were tested for the presence of *Arcobacter* species. Suspected colonies were subjected to biochemical tests and identified by phenotypic methods. In addition, antimicrobial susceptibility testing was performed using the disk diffusion method. *Arcobacter* spp. were also directly detected by multiplex-PCR.
Findings: Out of 230 samples, 20 samples (8.69%) were positive in culture method, and 44 samples (19.13%) were positive in PCR method, all culture-positive samples were also positive in PCR method. Rep-PCR indicated 14 different rep types among *Arcobacter* spp. isolated from patients with gastroenteritis. All *Arcobacter* isolates were resistant to cefazolin, ceftazidime, and nalidixic acid. The isolates showed high susceptibility to tetracycline, gentamicin, ampicillin, amikacin, meropenem, erythromycin, and ciprofloxacin.
Conclusions: To the best of our knowledge, this is the first study conducted in Iran to isolate *Arcobacter* spp. from patients with gastroenteritis. The results indicate that *Arcobacter* spp. are one of the main causes of acute diarrhea in humans. The research outcomes show that *Arcobacter* spp. could be considered as the etiology of gastrointestinal infections in humans.

Keywords: Watery diarrhea, *Arcobacter*, PCR, Prevalence

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Introduction

Arcobacter spp. are Gram-negative, rod-shaped, non-spore forming, curved, motile bacteria. The genus *Arcobacter* is a member of the *Campylobacteraceae* family. The most significant difference between *Arcobacter* and *Campylobacter* species is the ability of *Arcobacter* spp. to grow under aerobic conditions and at low temperatures (15-25 °C) [1]. *Arcobacter* spp. have been isolated from many sources such as water, vegetables, foods of animal origin, food-processing equipment, domesticated and wild animals, primates, and birds [1-4].

The genus *Arcobacter* currently includes 26 species [4-6]. *A. cryaerophilus*, *A. skirrowii*, and particularly *A. butzleri* are associated with human diseases [7-10]. *A. butzleri* and *A. cryaerophilus* are predominantly associated with enteritis and bacteremia in humans [11, 12]. However, *A. skirrowii* is rarely isolated from humans. The clinical symptoms of *Arcobacter* infections are usually similar to those of campylobacteriosis, such as abdominal cramps and watery diarrhea [13]. However, continuous and watery diarrhoea has been reported to be more common in *Arcobacter* infections [11, 14].

Objectives: Several studies have reported the isolation of *Arcobacter* spp. from poultry and slaughterhouse samples in Iran [13], but as far as we know, no such studies have been conducted on *Arcobacter* species in human samples. Therefore, this study aimed to investigate the prevalence of *Arcobacter* species in human infectious diarrheal stool samples. Isolates were recognized at the species level using Multiplex PCR and then genotyped by repetitive sequence-based polymerase chain reaction (rep-PCR).

Material and Methods

Sample Collection: In this study, infectious diarrheal stool samples were collected from 230 patients admitted to educational hospitals and medical centers affiliated to Arak University of Medical Sciences over a two-month period from July to September 2016. None of the patients enrolled in this study received antibiotics for at least 7 days prior to sampling. The study questionnaire was completed after obtaining written consent from patients or their parents/ guardians. The study protocol No1395.62. was ratified by the Ethics Committee of Arak University of Medical Sciences.

Culture and Bacterial identification: In the laboratory, 1 mL of each specimen was inoculated into 9 mL of *Arcobacte*r broth (Ibresco, Iran) supplemented with CAT (cefoperazone, amphotericin B, and teicoplanin; Ibresco, Iran), then enrichment broths were incubated (48 hrs, 28°C) in candle jars. After incubation at 28°C for 48 hrs in candle jars, 6 to 8 drops of each enrichment broth was pipetted onto a membrane filter with 0.45 µm pore size and 25 mm diameter (filter-bio, China), placed on a *Brucella* agar plate (Ibresco, Iran) supplemented with 5% (v/v) sheep blood .The filtration was performed for 45 min at room temperature, and after removing the filter, the plates were incubated at 28ºC for 48 hrs under a candle jar. Suspected colonies (pinpointed, translucent, and watery colonies) were selected from each plate and then subcultured on *Brucella* agar. Pure cultures were confirmed by biochemical tests. The strain *A. butzleri* ATCC 49616 was used as a positive control [41].

Phenotypic characterization: The isolates were characterized phenotypically. All the isolates were checked by Gram staining, catalase and oxidase tests, H_2S production, growth at different temperatures under aerobic and microaerobic conditions, growth in the presence of NaCl (2% and 3.5%), growth on MacConkey agar, nitrate reduction test, and susceptibility to nalidixic acid (30 µg per disk) and cephalothin (30 µg per disk).

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DNA Extraction and PCR: About 5 mL of each diarrheal specimen was added to phosphate-buffered saline (PBS) and stored at -20°C. DNA was extracted from stool samples using the Stool DNA Isolation Mini Kit[1] (YTA, Iran). Genomic DNA was extracted from all *Arcobacter*-positive bacterial colonies (n=20) by genomic DNA Extraction Mini Kit[2] (YTA, Iran). The concentration of each DNA was specified spectrophotometrically at 260 and 280 nm. The extracted DNA samples were adjusted to 20 mg/µL and stored at -20 °C until used in PCR analysis. PCR assay was directly conducted on DNA extracted from stool samples.

Genus-specific PCR: For genus-specific PCR, Arc1 and Arc2 primers targeting a section of the 16S rRNA gene were used. PCR amplifications were performed in a final volume of 25 µL. PCR reaction mixtures consisted of 3 mL of template DNA, 12.5 µL of PCR Master Mix Red (1.5 mM $MgCl_2$; Ampliqon, Denmark), and 0.7 µL (10 pmol) of forward and reverse primers (CinnaGen, Iran) shown in Table 1. The volume of the reaction mixtures reached 25mL using distilled deionized (DI) water. PCR thermal cycling was performed in a gradient thermocycler under the following conditions: an initial denaturation step at 94°C for 5 min, followed by 28 cycles of denaturation at 94°C for one min, annealing at 52.7°C for 55 seconds, extension at 72°C for 55 seconds, and a final extension step at 72°C for 8 min. PCR products were separated by electrophoresis on 1.5% agarose gel and stained with 1% safe stain[3] (CinnaClon, Iran). The bands were visualized and recorded in a gel documentation system. The DNA of the reference strain was used as a positive control, and DI water was used as a negative control in PCR experiments [41].

Separation of *Arcobacter* Isolates by multiplex-PCR at the Species Level (Species-specific m-PCR): To differentiate *Arcobacter* strains at the species level, primers and PCR conditions specific to *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus* were utilized for *Arcobacter*-positive samples. The sequences of multiplex-PCR primers were targeted to three *Arcobacter* species 16S rRNA and 23S rRNA genes, the selected primers amplify a 401 bp fragment of *A. butzleri*, a 257 bp fragment of *A. cryaerophilus*, and a 198 bp fragment of *A. skirrowii*. Details of these primers are given in Table 1. The reaction mixtures and temperature gradients were set according to genus-specific PCR.

REP-PCR and electrophoresis: Rep-PCR was performed in a total volume of 25 mL of the prepared PCR mixture, consisting of 2 µL of template DNA, 12.5 µL of Taq DNA Polymerase Master Mix Red (1.5 mM $MgCl_2$; Ampliqon, Denmark), and 1.5 µL (10 pmol) of $(GTG)_5$ primer (5'-GTG GTG GTG GTG GTG-3') (CinnaGen, Iran). The volume of the reaction mixtures reached 25 µL using sterile water in molecular grade. DNA amplification was performed under the following conditions: an initial denaturation step at 94°C for 5 min; followed by 27 cycles of denaturation at 94°C for 1 min, 51°C for 1 min, and 65°C for 1 min; and a final extension step at 65°C for 8 min**.** PCR products were separated on 1.5% agarose gel in 0.5× TAE buffer at 80 V for 2 hrs. The gel was analysed by visual detection of various DNA profiles under UV light to detect DNA polymorphisms among the isolates.

All rep-PCR profiles were examined using GelCompar II® software (Version 6.6.11, Applied Maths BVBA, Kortrijk, Belgium). Gel images were normalized by aligning the size marker strips on each gel. The optimization setting and band position tolerance were 1%. The similarity of the band patterns was calculated using Pearson's correlation

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3. EP5081

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coefficient, and then the patterns were clustered using a dendrogram generated by unweighted pair group method with arithmetic mean (UPGMA) according to the rep-PCR results. Isolates with a similarity value more than 94% were placed in the same rep-PCR type [33].

Susceptibility testing: *Arcobacter* isolates were evaluated for susceptibility to 18 commercially available antibiotic disks using the disk diffusion technique. Suspensions of 0.5 McFarland standard turbidity were prepared from fresh culture of *Arcobacter* isolates in Mue ller-Hinton broth. Each suspension was inoculated onto a 150 mm Mueller-Hinton agar plate (Ibresco, Iran) with 5% sheep b lood using a sterile swab. The agar surfac es were allowed to dry, and antimicrobial disks were placed on the plates. The plates were incubated at 30°C for 48 hrs in a candle jar. The inhibition zones were measured using a scaled ruler in millimeter [13]. *Escherich ia coli* ATCC 25992 was used as a control st rain. The antibiotic disks and their conce ntrations were as follows: chloramphenicol (C, 30 µg), meropenem (TMP, 30 µg), n alidixic acid (NA, 30 µg), erythromycin (E, 15 µg), ciprofloxacin (CP, 15 µg), gentamicin (GN, 10 µg), amikacin (AK, 30 µg), tetracy cline (TE, 30 µg), cefazolin (CZ, 30 µg), am picillin (AM, 10 µg), and ceftazidime (CA Z 30 µg). Since there is no recommendati on of breakpoint values for *Arcobacter* strains, the Clinical and Laboratory Stan dards Institute guideline (M100–S26) for *Enterobacteriaceae* was used for all antibiotics (CLSI 2010, M45-A2) [23, 24].

Sequencing: The produced amplicons were sent for sequencing. The sequencing was carried out with the ABI Applied Biosystem machine, Model 3730XL (Macrogene, South Korea).

Findings

Isolation and identification: Out of a total of 230 samples, 20 samples (8.69%) were positive for the presence of *Arcobacter* species using culture method (Fig.1). The prevalence of *A. butzleri* (14 cases) among the samples was higher than that of *A. cryaeophilus* (4 cases) and *A. skirrowii* (2 cases). These results were confirmed by biochemical assays and PCR (Table 2). All the isolates were positive for oxidase and catalase production and nitrate reduction but negative for H_2S production and urease activity. The isolates showed variable growth rates on MacConkey agar in the presence of NaCl. All the isolates were susceptible to nalidixic acid and resistant to cephalothin. In PCR method, 44 samples (19.13%) were positive for the presence of *Arcobacter* species (Fig. 2). Among which, the prevalence of *A. butzleri* was more (11.3%, 26 of 230) compared to *A. cryaerophilus* (5.6%, 13 of 230) and *A. skirrowii* (2.17%, 5 of 230) (Table 2).

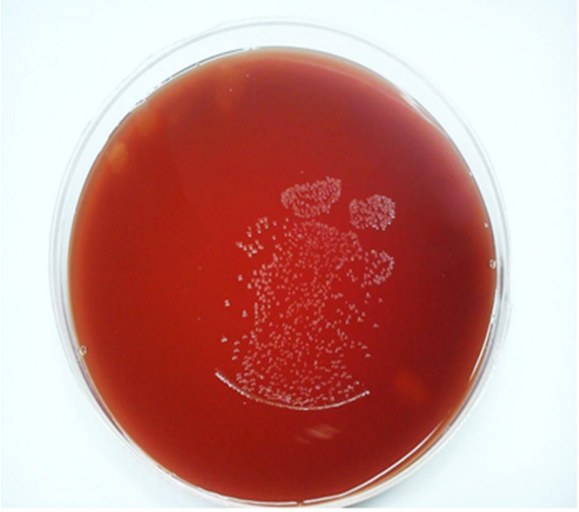

Figure 1) *Arcobacter* colony morphology on *Brucella* agar plates

Clinical features: *Arcobacter* species were isolated from 44 patients, including 28 children and 16 adults. The most common symptoms in all patients were abdominal pain with cramps, acute diarrhoea, and occasionally fever and nausea. Diarrhea was watery in 10 adult and 18 pediatric patients. In 11 pediatric and three adult patients, vomiting was accompanied by diarrhea. By microscopic examination (10X

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Table 1) Nucleotide sequences used as primers in the PCR reaction to identify *Arcobacter* genus and *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* species

Name of Primer	Sequence (5` to 3`)	Target Gene	Product Size (bp)	Reference
Arc 1 Arc 2	AGAACGGGTTATAGCTTGCTAT GATACAATACAGGCTAATCTCT	*16SrRNA*	181	(Gonzalez et al., 1999)
Butz Arco	CCTGGACTTGACATAGTAAGAATGA CGTATTCACCGTAGCATAGC	*16SrRNA*	401	(Houf et al. 2000)
Cry1 Cry2	TGCTGGAGCGGATAGAAGTA AACAACCTACGTCCTTCGAC	16SrRNA	257	(Houf et al., 2000)
SkiR ArcoF	TCAGGATACCATTAAAGTTATTGATG GCYAGAGGAAGAGAAATCAA	23SrRNA	198	(Douidah et al.2010)

Table 2) Comparison of sensitivity of culture and PCR methods in detecting *Arcobacter* species

Species	Culture N (%)	Polymerase Chain Reaction (PCR) N (%)
A. butzleri	14 (6.08)	26 (11.3)
A. cryaerophilus	4 (1.73)	13 (5.6)
A. skirrowi	2 (0.87)	5 (2.17)
Total	20 of 230 (8.69)	44 of 230 (17.3)

Table 3) Clinical features of patients infected with *Arcobacter* spp.

Clinical Features	Adult Patients (N=16)	Pediatric Patients (N=28)
Abdominal pain	11	28
Fever	5	18
Nausea	12	15
Vomiting	3	11
Watery diarrhea	10	18
Mucus in stool	6	10
Erythrocytes in stool	0	2
Leukocytes in stool	8	11
Clinical Status		
Ambulatory	16	23
Hospitalized	0	5

magnification), leukocytes and mucus were observed in the stool samples (Table 5). The clinical features of the patients are given in Table 3.

REP-PCR: A total of 20 *Arcobacter* isolates were examined, and 14 rep types were determined, including six common types and eight single types. All *A. butzleri* isolates (14 cases) were placed in three common types (A_3, A_9, and A_{13}) and eight single types. Also, four *A. cryaerophilus* isolates were placed in two common types (A_1 and A_2). In addition, two *A. skirrowii* strains

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Table 4) Resistance rates of *Arcobacter* spp. to antimicrobial agents

Antimicrobial Agent	*A. butzleri* (%) (N=14)	*A. cryaerophilus* (%) (N=4)	*A. skirrowii* (%) (N=2)
Amikacin	0(0)	0(0)	2(100)
Ampicillin	0(0)	0(0)	0(0)
Cefazolin	14(100)	4(100)	2(100)
Ceftazidime	14(100)	4(100)	2(100)
Gentamicin	0(0)	0(0)	0(0)
Ciprofloxacin	2(14.28)	0(0)	0(0)
Meropenem	0(0)	0(0)	0(0)
Nalidixic acid	14(100)	4(100)	2(100)
Tetracycline	0(0)	0(0)	0(0)
Erythromycin	3(21.42)	0(0)	0(0)
Chloramphenicol	14(100)	1(25)	0(0)

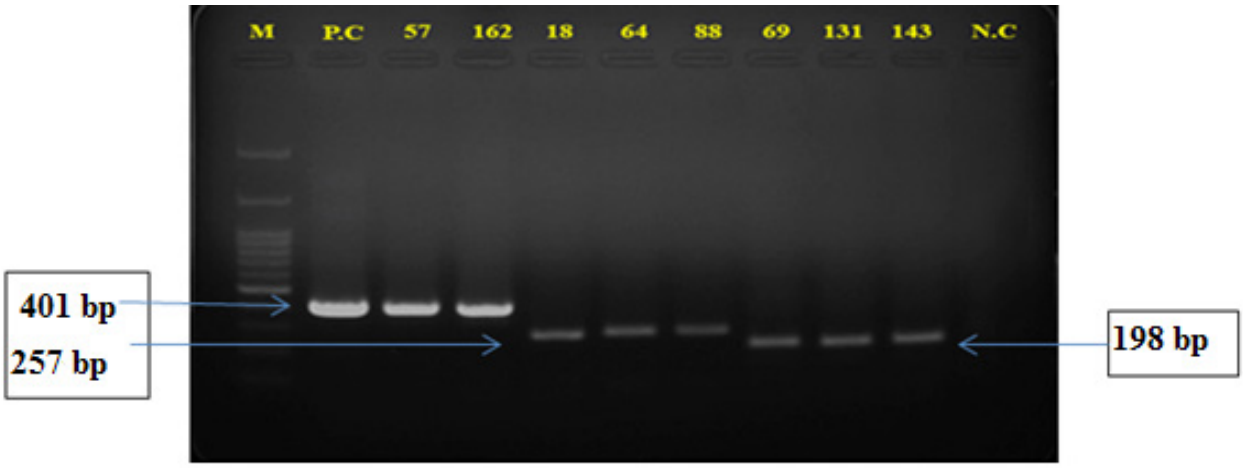

Figure 2) Detection of *Arcobacter* isolates at the species level by multiplex-PCR
Lanes: M: size marker (GeneRuler 100 bp DNA ladder; Fermentas), Lane 1: positive control *A. butzleri* ATCC 49616, Lanes 2–3: *A. butzleri* isolates, Lanes 4-6: *A. cryaerophilus* isolates, Lanes 7-9: *A. skirrowii* isolates, Lane 10: negative control (sterile distilled water)

showed a similar common type (A_{14}) (Fig. 3).

Antibiotic susceptibility test: All of the 20 *Arcobacter* isolates were resistant to one or more antimicrobial agents. The antimicrobial susceptibility testing results of the isolates are shown in Table 4. All *Arcobacter* isolates were found to be resistant to cefazolin, ceftazidime, nalidixic acid, and chloramphenicol (except two isolates of *A. cryaerophilus*) and susceptible to tetracycline, gentamicin (except two isolates of *A. skirrowii* and one isolate of *A. cryaerophilus*), ampicillin, amikacin (except two isolates of *A. skirrowii*), meropenem (except two isolates of *A.skirrowii*), erythromycin (except three isolates of *A. butzleri*), and ciprofloxacin (except two isolates of *A. butzleri*).

Sequencing: Sequencing results were analysed using Chromas, Mega 4.0, Blast, and Blat software (Fig. 4).

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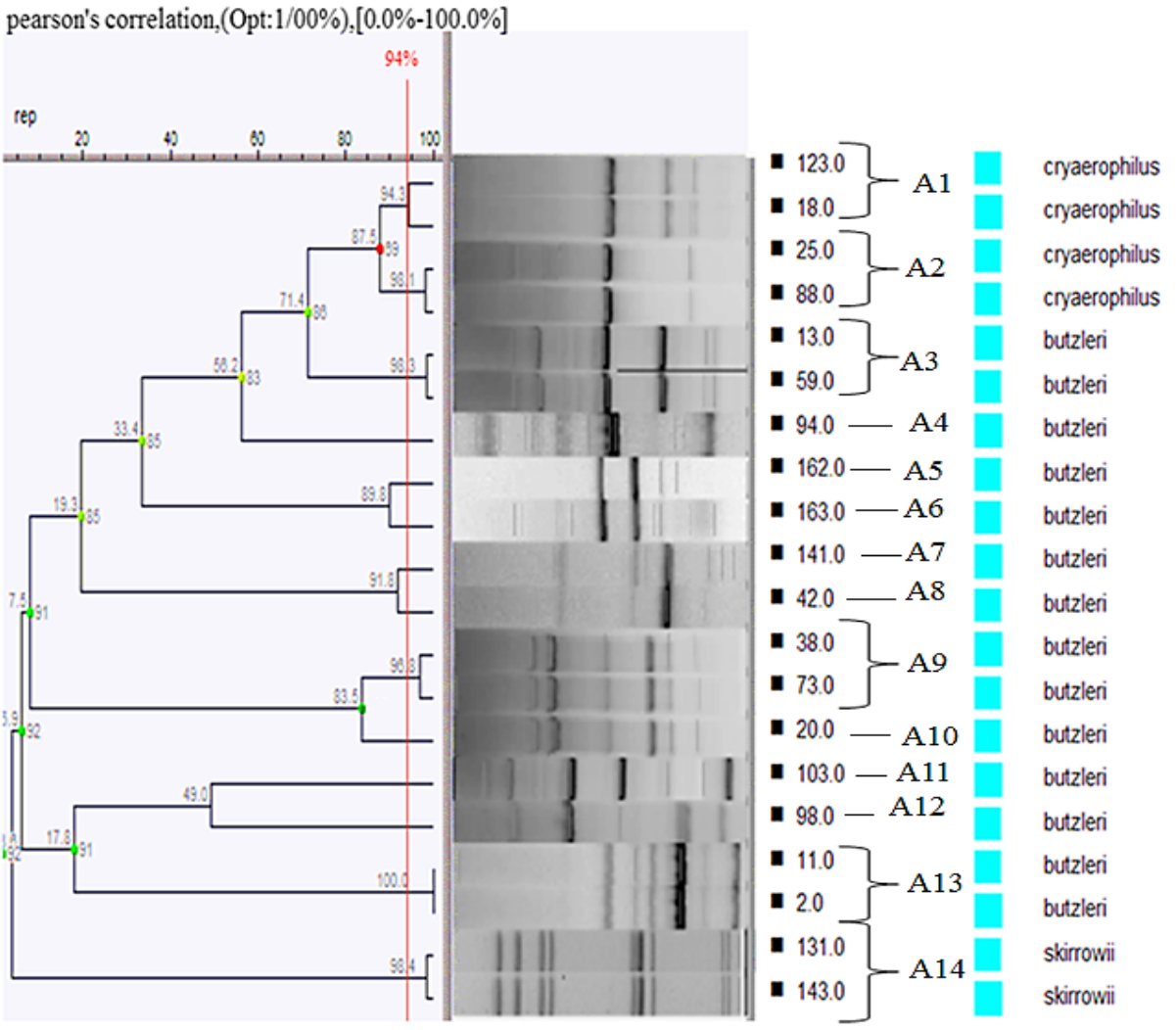

Figure 3) Numerical analysis of rep patterns of *Arcobacter* strains. The clustering of *Arcobacter* isolates is based on Pearson's correlation coefficient and the dendrogram constructed using UP-GMA with a tolerance of 1%. Isolates with a similarity value higher than 94% were considered in the same rep-PCR type.

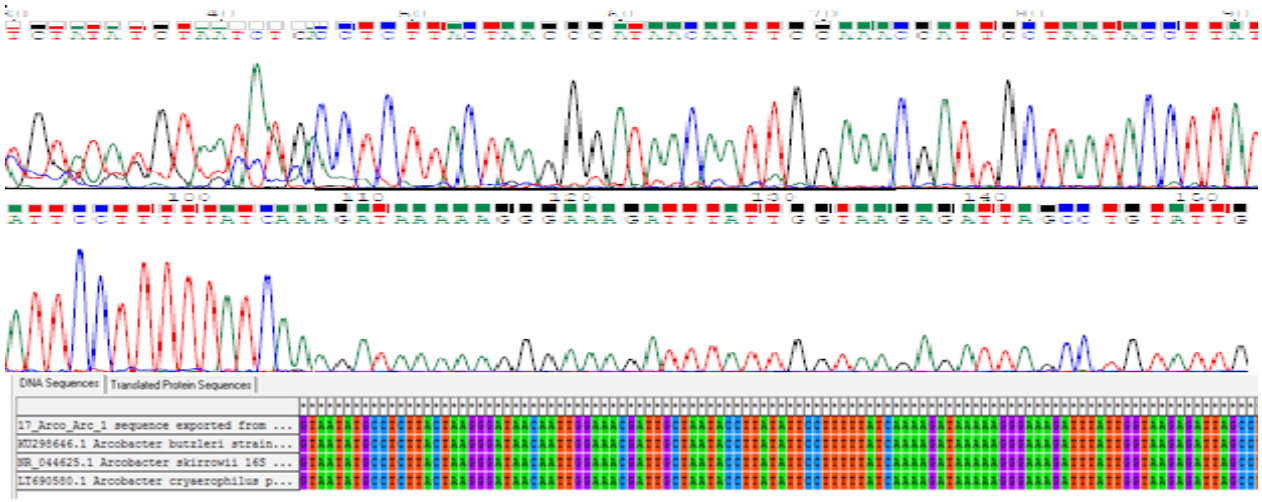

Figure 4) Nucleotide sequences obtained from the sequencing of clinical samples (Chromas and Mega4 software)

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Table 5) Demographic and clinical characteristics of *Arcobacter* infection

		Antibiotic Susceptibility											Clinical Features										
REP Type	Bacterial Species	Chloramphenicol	Nalidixic Acid	Tetracycline	Erythromycin	Ampicillin	Amikacin	Cefazolin	Ceftazidime	Gentamicin	Ciprofloxacin	Meropenem	Leukocytes in stool	Vomiting	Nausea	Fever	Abdominal pain	Type of Diarrhea	Reception	Contact Chicken	Consumption of vegetables	Place of Illness	Age	Gender
A_1	*A.cryaerophilus*	I	R	S	S	S	S	R	R	R	R	S	+	-	+	+	+	**2**	Outpatient	+	-	City	12	Male
A_1	*A.cryaerophilus*	S	R	S	S	S	S	R	R	R	S	S	+	-	-	-	+	1, 2	Outpatient	-	-	Village	1.8	Male
A_2	*A.cryaerophilus*	R	R	S	S	S	S	R	R	S	S	S	-	-	-	+	+	**2**	Outpatient	-	+	City	7	Male
A_2	*A.cryaerophilus*	S	R	S	S	S	S	R	R	S	S	S	-	-	+	-	+	**2**	Outpatient	-	+	City	51	Male
A_3	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	-	-	-	-	+	**2**	Outpatient	-	-	Village	49	Male
A_3	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	+	-	-	+	+	**1**	Hospitalization	-	-	Village	11 mon	Female
A_4	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	-	+	+	+	+	**2**	Outpatient	-	-	City	2.1	Female
A_5	*A.butzleri*	R	R	S	R	S	S	R	R	S	S	S	+	-	-	+	+	**1**	Outpatient	-	-	City	4.2	Female
A_6	*A.butzleri*	R	R	S	R	S	S	R	R	S	S	S	-	-	-	-	+	**1**	Outpatient	-	-	City	3	Female
A_7	*A.butzleri*	R	R	S	R	S	S	R	R	S	S	S	-	-	-	+	+	**2**	Outpatient	-	-	City	5	Female
A_8	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	+	-	-	+	+	**1**	Outpatient	-	-	City	3.8	Female
A_9	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	-	-	+	-	+	**1**	Outpatient	+	-	City	21	Female
A_9	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	-	-	-	+	+	**1**	Outpatient	-	-	City	4.6	Male
A_{10}	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	-	+	-	+	-	**1**	Outpatient	+	-	City	29	Male
A_{11}	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	+	+	+	-	+	**1**	Hospitalization	-	-	City	3.9	Female
A_{12}	*A.butzleri*	R	R	S	S	S	S	R	R	R	S	S	-	-	-	+	+	**1**	Hospitalization	-	-	City	1.9	Male
A_{13}	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	-	-	-	+	+	**2**	Outpatient	-	+	City	5	Male
A_{13}	*A.butzleri*	R	R	S	S	S	S	R	R	S	S	S	-	+	+	+	+	**1**	Outpatient	-	+	City	35	Male
A_{14}	*A.skirrowii*	S	R	S	S	S	R	R	R	S	R	R	+	+	-	+	+	**1**	Outpatient	-	-	City	1.1	Male
A_{14}	*A.skirrowii*	S	R	S	S	S	R	R	R	S	S	R	+	+	+	-	+	**1**	Hospitalization	-	-	City	5.4	Male

1: watery diarrhea, 2: mucoid diarrhea, S: sensitive, R: resistant, I: intermediate

Discussion

In this study, the prevalence of *Arcobacter* spp. in diarrheic stool samples was examined using culture and PCR methods; in addition, genotyping and antimicrobial susceptibility testing were carried out for the isolated strains. *Arcobacter* species have been isolated from symptomatic and asymptomatic people in many countries [10]. Some *Arcobacter* species have been isolated from the feces of patients with/without diarrhea, occasionally accompanied by bacteremia, endocarditis, and peritonitis [2, 8, 9]. The incidence of *Arcobacter* in human infections has been underestimated, especially due to the use of inappropriate detection and typing methods in stool culture [15]. *A. butzleri* is a species often observed in people with diarrhea.

The prevalence of *Arcobacter* isolates in this study was determined to be 19.13%

[DOI: 10.52547/iem.8.2.107]

by molecular method and 8.69% by culture method.

In the last decade, numerous studies conducted on the isolation and identification of *Arcobacter* spp. in different countries have reported variable prevalence rates. The prevalence rate obtaind for *Arcobacter* species in this study by PCR method was higher than the prevalence rates reported by Fernandez et al. (2015) in Chile (3.6%) [3], Samie et al. (2007) in South Africa (9.12%) [8], and Ramees et al. (2014) in India (11.76%) [17] but lower than the prevalence rates found by Fera et al. (2010) in Italy (46.46%) [18] and Webb et al. (2016) in Canada (60%) [19]. In all the mentioned studies, including the present research, the most prevalent species was *A. butzleri*. Also, the prevalence rate obtained by culture method in this study was higher than the results reported in South Africa (0.1%) [20] and Thailand (2.4%) [21]. In a study performed in India [17], the prevalence rate of *Arcobacter* strains isolated from human diarrheic stool samples was 2.67% using culture method, whilst 4% of the samples were positive in PCR screening test after enrichment. In another study conducted by Fera et al. (2010) in Italy [18], the prevalence rate of *Arcobacter* isolates was determined to be 3 and 46.46% by culture and PCR methods, respectively. In Canada in 2016 [19], the prevalence rate of *Arcobacter* isolates was determined to be 8.0% by culture method and 60% by PCR. In contrast, in a study conducted in Chile in 2015 [3], the prevalence rate of *Arcobacter* isolates was determined to be 7.0% by culture method and 4.1% by molecular methods. Mohan et al. (2014) also reported that the prevalence rate of *Arcobacter* spp. in human stools was 2% by cultural isolation and 2.00% by PCR screening [22].

By enrichment method in *Arcobacter* broth and then transfer onto *Arcobacter* selective medium, the prevalence of *Arcobacter*-positive samples among human stool samples has been reported to be 1.31% in Belgium and 0.9% in New Zealand [23]. Kayman et al. (2012) in Turkey examined 3287 stool samples, of which nine (0.3%) samples were positive for *Arcobacter* species; in their study, a modified CCDA-Preston medium was used to isolate *Arcobacter* spp. [24].

In the majority of these studies, the plates were incubated at 37°C for 2 to 3 days, or often for 3 to 4 days in microaerobic conditions, but in the current study, *Brucella* agar medium was used and incubated at 28 °C for 48 hrs in the candle jar.

Discrepancies in isolation rates may be due to the use of different isolation methods. Some of the factors influencing isolation rate are as follows: the sensitivity of isolation methods, test conditions (optimum incubation time, temperature required, type of culture medium, and atmosphere), study population, study area, diet, the degree of exposure to natural reservoirs of *Arcobacter* species, the immunity level of the studied population, living conditions, drinking water status, nutritional habits especially nutrition containing poultry, and the relationship with animals and the environment such as exposure to pets [25-28].

In the current study, *Arcobacter* spp. were detected in 8.68% of patients' stool cultures. All patients had gastroenteritis, and their clinical and laboratory features (Table 3) were similar to the results reported by Kayman et al. (2012) [24], Prouzet-Mauléon et al. (2006) [16]. and Vandenberg et al. (2004) [29]. The age range of pediatric and adult patients was 2-7 and 19-65 years, respectively. The main symptoms of *Arcobacter* infection were nausea, abdominal pain and cramps, acute diarrhea, and sometimes fever. In a study conducted in Turkey in 2012, the most common symptoms were nausea, abdominal pain, and fever [24]. In another study in France in 2006, severe diarrhea, abdominal pain,

[DOI: 10.52547/iem.8.2.107]

and fever were the most common disease symptoms [16]. The most common symptoms in children were abdominal pain, diarrhea, fever, and nausea, while in adults, the most common symptoms were diarrhea and nausea.

Genotyping of *Arcobacter* isolates has been used to determine the molecular epidemiology of *Arcobacter* spp. isolated from a variety of sources such as birds [30], cattle [31], and food [32]. It has been specified that this microorganism has a large number of subtypes. For example, in a study in Turkey, nine isolates typed as *A. butzleri* were found to have nine different genotypes using ERIC-PCR [24]. In another study in Thailand in 2013, 27 patterns were obtained for 33 isolates using rep-PCR [33]Xml. Subtyping of *Arcobacter* isolates in another study in India using ERIC-PCR revealed a great degree of heterogeneity among the isolates with 18 different subtypes for 27 *A. butzleri* isolates and 14 different subtypes for 22 *A. cryaerophilus* isolates [34].

In another study in India, the analysis of 16 *A. butzleri* isolates revealed 14 ERIC-PCR and 15 rep-PCR patterns. In addition, the analysis of 13 *A. cryaerophilus* isolates resulted in 13 ERIC-PCR and 12 rep-PCR patterns, representing the genetic diversity of *Arcobacter* species [35]. The genotyping results of *Arcobacter* species by rep-PCR in the present study resulted in 14 different rep types, indicating a high genetic diversity among 20 isolates with no dominant pattern. Genetic diversity among *Arcobacter* isolates observed in the present and previous studies suggests that there are multiple resources of *Arcobacter* contamination in the environment.

Extended use of antibiotics could lead to the development and spread of antibiotic-resistant bacteria in humans and animals [36]. Although *Arcobacter* strains have been isolated from human infections since 1991, so far no standardized procedures have been established to determine their antibiotic susceptibility patterns. However, several studies have been conducted on clinical cases to determine their antibiotic susceptibility pattern [23, 24, 37, 38]. In the present study, all *Arcobacter* isolates were resistant to cefazolin, ceftazidime, and nalidixic acid. In addition, resistance to chloramphenicol (75%) was found to be high among *Arcobacter* species. In a study conducted in New Zealand (2012), 12 *A. butzleri* strains were isolated from diarrheic patients. All *Arcobacter* isolates were sensitive to ciprofloxacin, and most of them were also sensitive to erythromycin (92%) but less sensitive to tetracycline (67%) and ampicillin (50%) [23]. In Turkey, Kayman et al. (2012) investigated antibiotic susceptibility of nine *A. butzleri* strains isolated from patients with acute or chronic diarrhea and found that all nine isolates were resistant to ampicillin and susceptible to gentamicin, tetracycline, erythromycin, and ciprofloxacin [24]. In a meta-analysis performed in 2018, it was shown that resistance rates to penicillins and cephalosporins ranged from 69.3–99.2% and 30.5–97.4%, respectively. The total resistance to fluoroquinolones ranged from 4.3 to 14.0%, and the highest resistance was observed to levofloxacin. Resistance rates to antibiotics ranged from 10.7–39.8% for macrolides, 1.8-12.9% for aminoglycosides, and 0.8–7.1% for tetracyclines [37]. A study in Belgium in 2016 showed that *Arcobacter* strains isolated from human stool samples were susceptible to gentamicin (99%) and tetracycline (89%). Erythromycin (78%), ciprofloxacin (72%), and doxycycline (76%) exhibited moderate activity against *Arcobacter* spp. Only %9 of the strains were susceptible to ampicillin [39-40].

Differences in these studies results may be due to differences in the methods used in

[DOI: 10.52547/iem.8.2.107]

antibiotic susceptibility testing. Ciprofloxacin and erythromycin are the antibiotics of choice in the treatment of *Campylobacter* infections [41]. The current study results also showed that all *Arcobacter* isolates were susceptible to tetracycline, ciprofloxacin, and ampicillin. These antibiotics, therefore, could be used to treat disease(s) caused by *Arcobacter* species.

Most isolates of similar genotypes showed the same resistance patterns; however, in some cases, common-genotype strains showed different antimicrobial resistance patterns.

Given the taxonomic proximity of *Arcobacter* to *Campylobacter* species, the presence of resistance and decreased susceptibility to erythromycin and ciprofloxacin among *Arcobacter* isolates is a matter of concern because these antimicrobial agents are commonly prescribed for the treatment of *Campylobacteraceae* infections in humans, and resistance to them may have consequences for human health.

Considering the high intake of chicken meat among people, the prevalence of these bacteria in the human gastrointestinal tract may be due to various reasons, among them the consumption of incompletely-cooked poultry meat or contact with raw meat and remnants of chicken digestive tract while washing chicken carcasses could play an important role in the transmission of these bacteria. To conclude, this is the first study conducted in Iran to detect *Arcobacter* species in human stool samples using molecular and culture methods with practical implications for controlling this pathogen.

Conclusion

In this study, the prevalence of *Arcobacter* spp. in diarrheic stool samples was evaluated using culture and PCR methods. In addition, genotyping and antimicrobial susceptibility testing were carried out for the isolated strains. The prevalence of *Arcobacter* spp. was determined to be 19.13% by molecular method and 8.69% by culture method. This is the first study conducted in Iran to isolate *Arcobacter* spp. from patients with gastroenteritis. The results show that *Arcobacter* spp. are one of the main causes of acute diarrhea in humans.

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