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Original Article

The evaluation of antibiotic resistance genes of *Arcobacter butzleri* isolated from animal products, and chicken slaughterhouse sewage in Mazandaran province, Northern Iran

Pourabbasgholi, Z.[1]; Kaboosi, H.[2*]; Ghane, M.[3]; Khoshbakht, R.[4] and Ghiamirad, M.[5]**

[1]Ph.D. Student in Microbiology, Department of Microbiology, Faculty of Basic Sciences, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran; [2]Department of Microbiology, Faculty of Basic Sciences, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran; [3]Department of Microbiology, Faculty of Basic Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran; [4]Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran; [5]Department of Microbiology, Faculty of Basic Sciences, Ahar Branch, Islamic Azad University, Ahar, Iran

[*]***Correspondence:*** *H. Kaboosi, Department of Microbiology, Faculty of Basic Sciences, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran. E-mail: h.kaboosi@iauamol.ac.ir*

[**]***Co-correspondence****: M. Ghane, Department of Microbiology, Faculty of Basic Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran. E-mail: masood.ghane@iau.ac.ir*

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Abstract

Background: *Arcobacter butzleri*, the most common genus of the Campylobacter family, is considered an emerging zoonotic pathogen. **Aims:** This study aimed to evaluate *A. butzleri* from diverse sources, in order to determine the antibiotic resistance pattern of isolates and the frequency of some genes responsible for their antibiotic resistance. **Methods:** In this study, 425 samples were collected from different sources (chicken slaughterhouse sewage, poultry meat, beef, sheep meat, dairy products) during different seasons of 2020-2021. Suspicious colonies were confirmed using biochemical tests. Furthermore, the polymerase chain reaction technique was used to confirm the phenotypic results using the *16S rRNA* gene. The antibiotic resistance pattern of the isolates to 16 antibiotics were determined using the disk diffusion method. Also, the minimum inhibitory concentration (MIC) of their growth was detected using the tube dilution method in the presence of tetracycline, erythromycin, and gentamicin. **Results:** A total of 53 isolates of *A. butzleri* (12.5%) were isolated from (chicken slaughterhouse sewage=36, poultry meat=8, beef=4, sheep meat=5), which contain all three antibiotic resistance genes of *abu_0814* (90.57%), *OXA_464* (100%), and *gyrA* (83.02%). The findings of the present investigation showed the presence of *A. butzleri* in different sources and the high prevalence of antimicrobial resistance in the isolates. Nineteen isolates (36%) have extensive drug resistance and 34 isolates (64%) showed multi-drug resistance to the used antibiotics. **Conclusion:** The elevated level of antibiotic resistance observed in *A. butzleri* isolates originating from various samples suggests a significant use of antibiotics and a prevalent environmental contamination.

Key words: Animal products, Antimicrobial resistance, *Arcobacter butzleri*, Extensive drug resistance, Multi-drug resistance

Introduction

The genus *Arcobacter* was included in the family Campylobacteraceae in 1991 (Vandamme *et al*., 1991). *Arcobacter* has undergone a restructuring, resulting in its division into six distinct genera: *Arcobacter*, *Aliarcobacter*, *Halarcobacter*, *Malaciobacter*, *Poseidonibacter*, and *Pseudarcobacter* (Pérez-Cataluña *et al*., 2019a). Among the twenty-nine officially recognized *Arcobacter* species, they are now spread across these newly defined genera, with the species *Arcobacter butzleri* being reclassified as *Aliarcobacter butzleri* within the genus *Aliarcobacter* (now *Aliarcobacter butzleri*) belonging to the genus *Aliarcobacter* (Pérez-Cataluña *et al*., 2019b; Oren and Garrity, 2020). *A. butzleri* typically results in a self-limiting acute enteritis characterized by watery diarrhea, nausea, abdominal pain, and occasionally fever in individuals (Collado and Figueras, 2011; Ramees *et al*., 2017). The diarrhea is a result of the disruption of epithelial barrier function caused by alterations in tight-junction proteins and the stimulation of epithelial apoptosis (Bucker *et al*., 2009). An extensive research conducted over an extended period revealed that *A. butzleri* ranks as the fourth most prevalent Campylobacter-like microorganism identified in human fecal samples (Vandenberg *et al*., 2004). In exceptional instances, *A. butzleri* might lead to bacteremia. In the

case of animals, *A. butzleri* has been linked to the occurrence of enteritis and/or diarrhea in pigs, cattle, and horses; nevertheless, it has also been observed in the fecal matter of healthy animals (Van Driessche *et al*., 2003; Atabay *et al*., 2008). *A. butzleri* stands out as the most predominant *Aliarcobacter* spp. identified in food (Lehner *et al*., 2005). These bacteria have been recovered from animal-derived items like poultry meat, pork, and beef, as well as water sources, dairy products, milk, shellfish, and vegetables (Arias *et al*., 2011; Yesilmen *et al*., 2014; Fanelli *et al*., 2019). The ingestion of tainted food or water represents the most likely pathway of transmission to both humans and animals (Shah *et al*., 2012; Ferreira *et al*., 2016). Contact with domestic animals can serve as a potential route of transmission to humans, given that *A. butzleri* has been detected in the oral cavity of felines (Fera *et al*., 2009; Ramees *et al*., 2017). Consequently, *A. butzleri* is not solely an emerging pathogen responsible for foodborne illnesses and zoonoses, but it is also deemed a significant threat to human well-being (ICMSF, 2018; Chieffi *et al*., 2020). Nevertheless, the modes of horizontal and vertical transmission in animals have been documented (Ho *et al*., 2006). Previous research has predominantly focused on investigating the antibiotic susceptibility of *A. butzleri* phenotypically; however, in the last few years, the underlying antimicrobial resistance (AMR) genes have also been studied more closely (Parisi *et al*., 2019; Isidro *et al*., 2020). The excessive use of antimicrobial drugs in animal husbandry and the transfer of contamination through the release of wastewater have led to the evolution and spread of drug-resistant isolates. They have also caused the transfer of resistance genes to other medically important Gram-negative bacteria (Isidro *et al*., 2020). The increase in multidrug-resistant (MDR) strains, which are resistant to three or more categories of antimicrobial agents, occurs as a consequence of the incorrect use of antibiotics for either preventive purposes or the treatment of infectious diseases. However, there are limited studies on frequency, antibacterial susceptibility, and antibiotic-resistance genes in *Arcobacter* strains (Atabay and Corry, 1997). The present study aimed to isolate and identify *A. butzleri* from different sources, determining the antibiotic resistance pattern of isolates of this bacterium to different antibiotics and the frequency of some genes responsible for antibiotic resistance.

Materials and Methods

Samples of collection

In this study, 425 samples were collected from different sources, including chicken slaughterhouse sewage (297 samples), poultry meat (20 samples), beef (20 samples), sheep meat (20 samples), and dairy products (68 samples) during different seasons of 2020-2021. For sampling, sterile swabs (sterilized at 121°C for 15 min) were used. All the collected samples were placed in a container filled with ice. Finally, samples were transferred at 4°C to the laboratory of microbiology in Islamic Azad University, Tonekabon Branch, Iran.

Isolation of bacteria from collected samples

Arcobacter spp. was isolated using the standard culture technique (Atabay and Corry, 1997; Fanelli *et al*., 2019; Kim *et al*., 2019). To isolate bacteria, different samples were enriched in Preston broth containing Preston supplement (Biomark, India). Incubation was carried out at a temperature of 25°C for 24 h. Subsequently, the cultures were passed through filters with 0.45 µm pore size in completely sterile conditions. After 48 h, the filtered samples were cultured on the surface of CAMPY agar (Merck, Germany) containing defibrinated sheep blood and supplemental antibiotics such as Vancomycin and Polymyxin. Then, the cultures were incubated at 25°C for 48-72 h (Fanelli *et al*., 2019). The isolates were identified using Gram staining, motility test, growth at different temperatures and under aerobic and microaerophilic conditions, growth in Macconkey agar (Merck, Germany), and common biochemical tests such as catalase, oxidase, and urease tests. After the final confirmation, the isolated bacteria were kept at -20°C.

Evaluation of antibiotic susceptibility of isolates

To determine the pattern of antibiotic resistance of *A. butzleri* isolated from different samples, the disk diffusion test (Hudzicki, 2009) was used based on the standards of the Clinical and Laboratory Standards Institute (CLSI) (Humphries *et al*., 2021). Briefly, isolates were cultured on Preston broth at 25°C for 48 h. Then, a microbial suspension with a concentration of half McFarland was made using physiological serum. The prepared suspension was densely cultured using an L-shaped rod on the surface of Mueller Hinton agar (Merck, Germany). After placing the antibiotic discs on the surface of the culture medium, the plates were incubated at 25°C for 24-48 h. Finally, the diameter of the bacterial growth inhibition zone around each disc was measured using a caliper and the results were interpreted using CLSI standard tables.

Sixteen antibiotic disks of Penicillin (PEN, 10 u), Oxacillin (OXA, 10 µg), Amikacin (AMK, 30 µg), Ampicillin (AMP, 10 µg), Amoxicillin (AMX, 25 µg), Amoxicillin/Clavulanic acid (AMC, 20/10 µg), Cephalothin (KF, 30 µg), Chloramphenicol (CHL, 30 µg), Erythromycin (ERY, 15 µg), Gentamicin (GEN, 10 µg), Nalidixic acid (NAL, 30 µg), Nitrofurantoin (NIT, 300 µg), Tetracycline (TCY, 30 µg), Ciprofloxacin (CIP, 5 µg), Trimethoprim/Sulfamethoxazole (SXT, 1.25/23.75 µg), and Azithromycin (AZM, 15 µg) were selected to perform this study. All antibiotics were purchased from Padtan Teb Company (Iran). After the selection and screening of *A. butzleri* strains, the minimum inhibitory concentration against Tetracycline, Erythromycin, and Gentamicin antibiotics was determined by broth macrodilution tube method based on CLSI standards.

Design of primer

Three primers of *OXA_464*, *abu_0814*, and *gyrA*

were designed using the Gene Runner 6.5 software package. The primers were validated through cross-referencing with the GenBank database to confirm their lack of significant similarity to genetic sequences from non-*Arcobacter* species. The pairs of *16S rRNA* primers were used by Scullion *et al*. (2006) (Table 1).

Molecular identification of *A. butzleri* strains and antibiotic resistance genes

All isolates were characterized through the employment of polymerase chain reaction (PCR). Bacterial DNA was isolated using a DNA extraction kit for this specific procedure. To do so, bacterial DNA was extracted using a DNA extraction kit (Kiagen Teb Sadra Company, Iran). The *16S rRNA* gene and resistance genes of *OXA_464*, *abu_0814*, and *gyrA* were amplified using the specific primers (Table 1).

In addition, each solution of PCR reaction with a final volume of 20 µL included 10 µL of master mix (Hot Start TaqPolymerase, dNTP, buffer, MgCl), 1 µL of forward primer, 1 µL of reverse primer, 7 µL of distilled water and 1 µL of template DNA. For polymerization of the *16S rRNA* gene, initial denaturation of the DNA samples was performed at 94°C for 4 min. Then, 30 cycles of PCR reaction including 94°C for 1 min (denaturation), 58.5°C for 1 min (annealing), and 72°C for 90 s (extension) were performed. The final extension was performed at 72°C for 10 min. The PCR products were taken on 1.5% agarose gel for final analysis. Thus, 5 µL of the amplified DNA was transferred into each of the wells embedded in the agarose gel. The voltage was set to 75 V for 40 min. Finally, the gel was transferred into the UV transluminator (UV Doc-England) to observe the bands of PCR products. Therefore, to ensure the proper amplification of *16S rRNA* and *OXA_464*, *abu_0814*, *gyrA* gene in the PCR experiment, the obtained product was sent to Macrogen, South Korea for sequencing. All sequences data were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) to definitively identify each respective *16S rRNA OXA_464*, *abu_0814*, *gyrA* gene amplicon.

Multiplex PCR conditions and gel electrophoresis

PCR reactions were conducted in a 25 µL reaction mixture containing 3 µL template DNA, 10 µL of the Master mix (Hot Start TaqPolymerase, dNTP, buffer, MgCl), 1 µL of each of the primers, and 6 µL of distilled water. The PCR reactions were performed in a thermal cycler lab-cycler 48 (lab-cycler, Germany) with the following amplification conditions: a denaturation step at 94°C for 4 min; 35 amplification cycles: denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 90 s; and the final extension step at 72°C for 10 min. The PCR products were analyzed on 1.5% (w/v) TBE-agarose gel using a 100 bp ladder (BIO BASIC, Canada). The band patterns were analyzed in the gel documentation system (UV Doc-England). DNA from reference strains (*A. butzleri*, ATCC 49616) and sterile deionized water was used as positive and negative controls, respectively (Çelik and Otlu, 2020).

Results

Isolation and identification of *A. butzleri* from different sources

From 425 samples collected during different seasons of 2021-2020, 53 isolates of *A. butzleri* (12.5%) were detected in different sources (Table 2).

The highest rate of isolation was related to poultry meat (40%). The highest isolation rate of the bacterium from different sources was 45.28% in the fall while the lowest isolation rate (7.55%) was detected in the winter (Table 3).

Table 1: The sequence of primers used for the evaluation and identification of *A. butzleri* from different resources

Gene	5´ → 3´	Product size (bp)	Reference
Arco	F: AGAGATTAGCCTGTATTGTATC R: TAGCATCCCCGCTTCGAATGA	1200	Scullion *et al*. (2006)
gyrA	F: TGCTAAAATTGCAGATGTACCA R: AATTCCTTTTTCAGAAACTGTACG	212	This study
OXA_464	F: ATTCGCAAATGATGTGGAAC R: TCCCATAATTAAGCTCTTTTAG	362	This study
Abu_0814	F: AGTCGTTAGTTGCAATATCT R: ATTTGAACTTTTGTATCTGG	1026	This study

Table 2: Number of *A. butzleri* isolates based on sample type

Sample type	Positive	Negative	Total	Chi-square (X^2)	P-value
Chicken slaughterhouse sewage	36 (12.1%)	261 (87.9%)	297 (100%)	17.258	0.001
Poultry meat	8 (40.0%)	12 (60.0%)	20 (100.0%)	2.234	0.027
Beef	4 (20.0%)	16 (80.0%)	20 (100.0%)	14.312	0.001
Sheep meat	5 (25.0%)	15 (75.0%)	20 (100.0%)	3.808	0.001
Dairy products	0 (0.0%)	68 (100.0%)	68 (100.0%)	-	-
Total	53 (12.5%)	372 (87.5%)	425 (100.0%)	13.980	0.001

Table 3: The frequency of *A. butzleri* isolated in different seasons of the year

Season	Frequency	Chi-square (X^2)	P-value
Spring	18 (33.96%)		
Summer	7 (13.21%)		
Fall	24 (45.28%)	8.554	0.001
Winter	4 (7.55%)		
Total	53 (100.0%)		

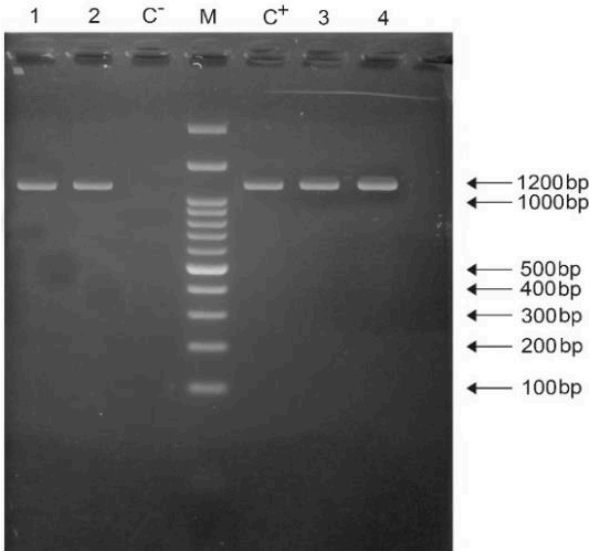

Fig. 1: Electrophoresis pattern of PCR for detection of *Arco* gene in *Arcobacter butzleri* isolates. M: Marker 100 bp, C+: Positive control, C-: Negative control, and Lanes 1, 2, 3, and 4: Amplification of *Arco* gene at 1200 bp

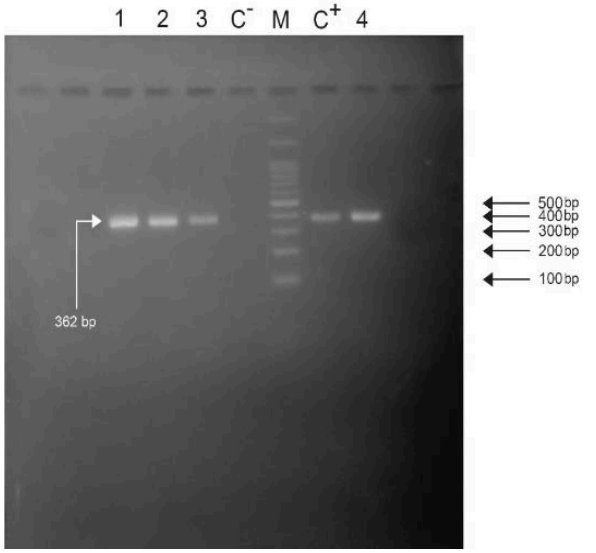

Fig. 2: Electrophoresis pattern of PCR for detection of *OXA_464* gene in *Arcobacter butzleri* isolates. M: Marker 100 bp, C+: Positive control, C-: Negative control, and Lanes 1, 2, 3, 4 and 5: Amplification of *OXA_464* gene at 362 bp

Authentication of *A. butzleri*

The isolates were confirmed as *A. butzleri* using genus-based PCR and species-specific multiplex PCR. The results of PCR and sequencing of *16S rRNA* genes confirmed the phenotypic results obtained in 53 isolates (Fig. 1).

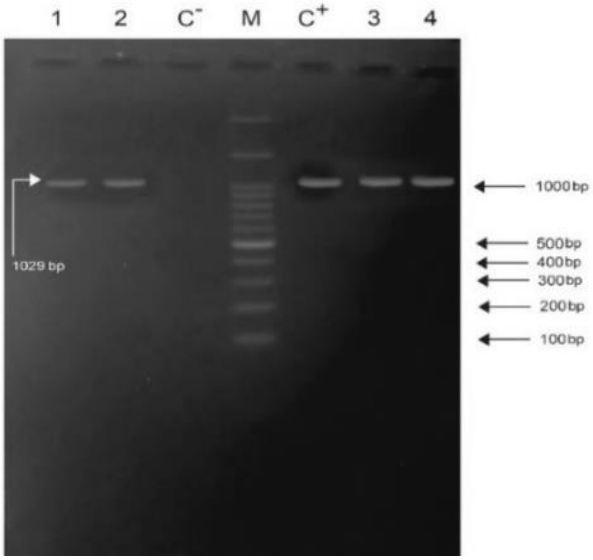

Fig. 3: Electrophoresis pattern of PCR for detection of *abu_0814* gene in *Arcobacter butzleri* isolates. M: Marker 100 bp, C+: Positive control, C-: Negative control, and Lanes 1, 2, 3 and 4: Amplification of *abu_0814* gene at 1029 bp

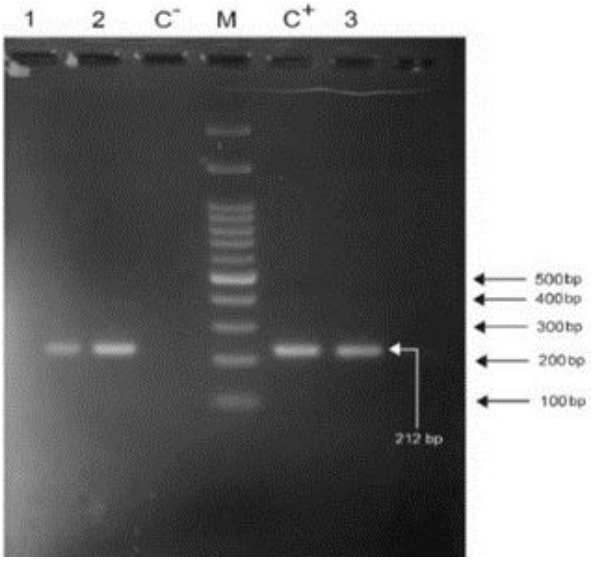

Fig. 4: Electrophoresis pattern of PCR for detection of *gyr*A gene in *Arcobacter butzleri* isolates. M: Marker 100 bp, C+: Positive control, C-: Negative control, and Lanes 1, 2 and 3: Amplification of *gyr*A gene at 212 bp

Evaluation of the presence of antibiotic-resistance genes in the isolates

The presence of *OXA_464*, *abu_0814*, and *gyrA* genes in *A. butzleri* was investigated using the PCR technique. According to the study results, the presence of the *OXA_464* gene was observed in all 53 isolates (100%) (Fig. 2). Also, 48 isolates (90.57%) were positive for the *abu_0814* gene (Fig. 3), and *gyrA* was detected in 44 isolates (83.02%) (Fig. 4). Finally, after examining all antibiotic resistance genes using the single-PCR method, the frequency of *abu_0814*, *OXA_464*, and *gyrA* in all isolates were studied by multiplex-PCR technique (Fig. 5).

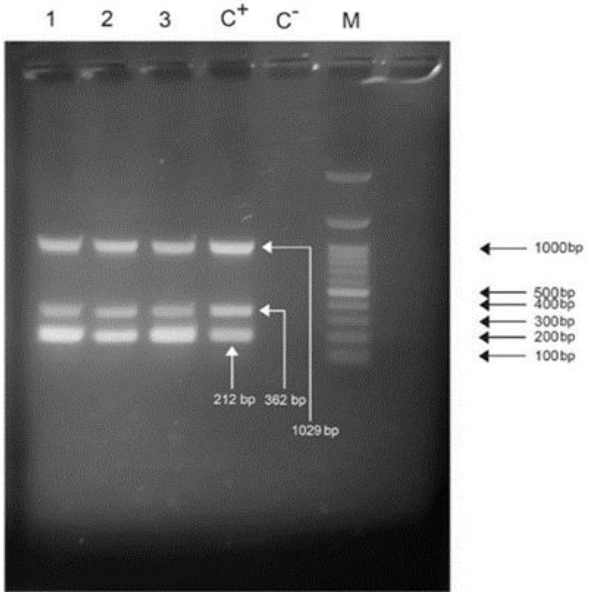

Fig. 5: Electrophoresis pattern of multiplex PCR for detection of *OXA_464*, abu_0814, and *gyr*A genes in *Arcobacter butzleri* isolates. M: Marker 100 bp, C+: Positive control, C-: Negative control, and Lanes 1, 2 and 3: Amplification of *gyrA*, *OXA_464*, and *abu_0814* genes at 212, 362, and 1029 bp, respectively

Sequencing analysis

The findings derived *16S rRNA*, *OXA_464*, *abu_0814*, and *gyrA* gene sequencing of PCR products were clear. There was 100% alignment agreement for all the samples using the BLAST alignment program. As shown in Table 4, BLAST analysis of the *16S rRNA*, *OXA_464*, *abu_0814*, *gyrA* gene sequence data for sample 1-4 indicated a 100% homology with the *A. butzleri* strain ED-1. Samples 5 indicated a 99% homology with the *A. butzelri* strain ED-1. Sample 6 was identified using the BLAST analysis of the sequencing data as *A. butzleri* strain ATCC 49616 (98% homology). Sample 7 was identified using the BLAST analysis of the sequencing data as *A. butzleri* strain 7h1h (100% homology). Sample 8 was identified using the BLAST analysis of the sequencing data as *A. butzleri* strain NCTC12481 (98% homology). Sample 9 was identified using the BLAST analysis of the sequencing data as *A. butzleri* strain JCR7715 (98% homology). Sample 10 was identified using the BLAST analysis of the sequencing data as *A. butzleri* strain P1100 (98% homology). The data concerning the comparison between phenotyping and genotyping for identifying the isolates revealed that the majority of genotyping results supported our phenotyping identification.

The frequency of *abu_0814*, *OXA_464*, and *gyrA* genes in the isolates was 90.57%, 100%, and 83.02%, respectively (Table 5).

Table 5: Frequency of antibiotic-resistance genes in *A. butzleri* isolates

Gene	Frequency	Chi-square (X_2)	P-value
OXA_464	53 (100.0%)		
abu_0814	48 (90.57%)	2.013	0.187
gyrA	44 (83.02%)		

Determining antimicrobial susceptibility pattern

All isolates showed resistance to Penicillin (100%), Ampicillin (100%), and Oxacillin (100%). Also, the levels of resistance to Trimethoprim/Sulfamethoxazole (68%), Ciprofloxacin (78%), Nalidixic acid (74%), Azithromycin (68%), and Amoxicillin (68%) were detected. Out of the 53 isolates tested, all isolates showed susceptibility to Gentamicin (100%) (Table 6). The MIC and MBC of 72% of the isolates to Tetracycline were ≥128 µg/ml and ≥256 µg/ml, respectively. All of the isolates (100%) had MIC ≥8 µg/ml and MBC ≥32 µg/ml to Gentamicin. Totally, 75% of the isolates had MIC ≥64 µg/ml and MBC ≥128 µg/ml to Erythromycin. Also, 19 isolates were detected as extensively drug-resistant (36%) and 34 isolates had multi-drug resistant (64%).

Discussion

Arcobacter species are globally recognized for their role in the etiology of acute gastroenteritis, worldwide (Zhang *et al*., 2019; Brückner *et al*., 2020; Chieffi *et al*., 2020). The primary mode of transmission of *Arcobacter* spp. to humans is through the ingestion of water and food that have been contaminated (Collado and Figueras, 2011; Zambri *et al*., 2019). Advancements in research regarding the frequency and virulence of *A. butzleri* and *A. cryaerophilus* have resulted in their classification as significant threats to human health by the International Commission on Microbiological Specifications for Foods (ICMSF, 2018).

Table 4: Comparison of phenotyping and genotyping identification

Number of samples	Phenotyping detection	Genotyping detection
1	*A. butzleri*	100% homology with the *A. butzleri* strain ED-1
2	*A. butzleri*	100% homology with the *A. butzleri* strain ED-1
3	*A. butzleri*	100% homology with the *A. butzleri* strain ED-1
4	*A. butzleri*	100% homology with the *A. butzleri* strain ED-1
5	*A. butzleri*	99% homology with the *A. butzleri* strain ED-1
6	*A. butzleri*	98% homology with the *A. butzleri* strain ATCC 49616
7	*A. butzleri*	100% homology with the *A. butzleri* strain 7h1h
8	*A. butzleri*	98% homology with the *A. butzleri* strain NCTC12481
9	*A. butzleri*	98% homology with the *A. butzleri* strain JCR7715
10	*A. butzleri*	98% homology with the *A. butzleri* strain P1100

Table 6: Antibiotic resistance pattern of *A. butzleri*

Antibiotic agent	Dick content	Interpretive categories and zone diameter breakpoint whole mm			R%	COD
		S	I	R		
Ampicillin	10 mg	17≥	14-16	13≤	100 R	AMP
Amoxicillin	25 mg	17≥	14-16	13≤	68 I	AMX
Amoxicillin-clavulanic acid	20/10 mg	17≥	14-17	13≤	75 I	AMC
Gentamycin	10 mg	15≥	13-14	13≤	100 S	GEN
Azithromycin	15 mg	13≥	14-16	12≤	68 R	AZM
Erythromycin	15 mg	23≥	14-22	13≤	75 S	ERY
Chloramphenicol	30 mg	18≥	13-17	15≤	73.5 I	CHL
Tetracycline	30 mg	15≥	12-14	12≤	68 I	TCY
Trimethoprim/Sulfamethoxazole	1.25/23.75 mg	16≥	11-15	10≤	68 R	SXT
Amicacin	30 mg	17≥	14-16	13≤	89 S	AMK
cephalothin	30 mg	23≥	20-22	19≤	57 I	CEP
ciprofloxacin	5 mg	12≥	16-20	15≤	78 R	CIP
nitrofurantoin	300 mg	17≥	15-16	12≤	67 S	NIT
Nalidixic acid	30 mg	19≥	14-18	14≤	74 R	NAL
Oxacillin	1 mg	18≥	14-18	12≤	100 R	OXA
penicillin	10 u	18≥	14-16	12≤	100 R	PEN

R: Resistant, I: Intermediate, and S: Sensitive

This study offers recent data concerning the prevalence and antibacterial resistance patterns of *A. butzleri*. Specimens were collected from diverse origins in Tonekabon, Iran.

Our findings indicated that the frequency of *A. butzleri* was observed, ranging from high to low, in poultry meat (40.0%), sheep meat (25.0%), beef (20.0%), chicken slaughterhouse sewage (12.1%), and dairy products (0.0%). Several studies investigated the presence of *Arcobacter* spp. in chicken meat. Similar to the results of the present study, Amare *et al*. (2011) identified *Arcobacter* spp. in 39% of fresh chicken meat. Di Noto *et al*. (2018) found *Arcobacter* spp. in 53.3% of the wing and carcasses of chickens. In another study conducted in Mexico by Scullion *et al*. (2006), the prevalence of *Arcobacter* spp. in chicken, beef, and raw milk samples were about 35%, 34%, and 46%, respectively. *A. butzleri* was the dominant species isolated from samples (Aski *et al*., 2016). The difference in the isolation rate of *Arcobacter* spp. may be due to different conditions in the research process including the geographical location, season of study, analysis methods, design of the study, types of used antimicrobial agents and their concentrations in culture mediums.

In the present study, the resistance rate of *Arcobacter* isolates to Ciprofloxacin, Nalidixic acid, and Azithromycin was 78%, 74%, and 68%. The increase in the rate of antibiotic resistance in Iran may be related to the excessive and arbitrary use of antibiotics. Also, the high resistance of pathogenic bacteria to Penicillins is usually associated with the presence of beta-lactamases. In the present study, almost all strains of *Arcobacter* isolates have *β*-lactam antibiotic resistance genes. It has been speculated that *A. butzlri* may be resistant to b-lactamase. Several studies reported that b-lactam resistance may be caused by the presence of three putative b-lactamases (AB0578, AB1306, and AB1486) identified in the RM4018 genome, which are enhanced by the occurrence of the lrgAB operon (ab0179 and ab0180) and may regulate tolerance to penicillin in *Staphylococcus* (Bayles, 2000; Groicher *et al*., 2000; Miller *et al*., 2007).

In the present study, the frequency of *abu_0814* gene in the isolates was reported as 90.57%. The presence of this gene corresponds to high resistance to beta-lactams. The presence of *abu_0814* can lead to resistance against beta-lactams in *Arcobacter* spp. and there was an agreement between the presence of this gene and the results obtained from Antibiogram and Whonet software.

The increase in the rate of resistance to Penicillin, Ampicillin, Oxacillin, Trimethoprim/Sulfamethoxazole, Nalidixic acid, Ciprofloxacin, and Azithromycin among *A. butzleri* isolates is a cause for concern (Aski *et al*., 2016). Van den Abeele *et al*. (2016), conducted a study that showed resistance to Ciprofloxacin is attributed to the development of resistance in *Arcobacter* strains due to mutations in the *gyrA* gene. In this study, by examining the frequency of *gyrA* in isolates and its relationship to their resistance to ciprofloxacin, it is possible to understand the relationship between mutations in *gyrA* and increased resistance to ciprofloxacin. Since high resistance levels of isolates to fluoroquinolones were shown in the present study, the results were consistence with the findings of other researchers (Houf *et al*., 2001). Several studies showed that the base mutation in the *gyrA* gene was associated with a higher level of resistance to ciprofloxacin (Webb *et al*., 2018; Hodges *et al*., 2021). In our study, 13 *A. butzleri* and 14 *A. cryaerophilus* isolates with *gyrA* (C254T) mutation were 100% phenotypically resistant to ciprofloxacin. Moreover, the genes aph (3')-IIIa and ant (6)-Ia were reported to correlate with resistance to kanamycin and streptomycin, respectively (Hormeño *et al*., 2018; Cho *et al*., 2020). All isolates showed sensitivity to Amikacin, Gentamicin, Erythromycin, and Nitrofurantoin. Also, 75%, 73.5%, and 68% of the isolates showed sensitivity to Amoxicillin/clavulanic acid, Chloramphenicol, and Tetracycline, respectively. It can be anticipated that the *Arcobacter* spp. is going to gain resistance to tetracycline.

This phenomenon can be attributed to the excessive use of antibiotics in the food chain of poultry due to the selective stress of antibiotics, the unavailability of other antibiotics, and the acquisition of resistance genes in the life cycle of these microorganisms. Resistance to Penicillin and Ampicillin is not surprising because these antibiotics are commonly used in selective media for the isolation of these organisms as well as in the treatment of *Campylobacter* infections (González *et al*., 2017).

Houf *et al*. (2001) evaluated 47 strains of *A. butzleri* isolated from different sources against several antimicrobial agents and found that most of the tested strains were resistant to Cefoperazone. Thus, specific attention should be paid to the use of Penicillin and Trimethoprim/Sulfamethoxazole, which are commonly used in the treatment of bacterial infections in humans and animals. In the present study, variable sensitivity to amoxicillin, clavulanic acid, chloramphenicol and tetracycline should also be considered when using these antibiotics against *Arcobacter* spp. infections. Although most of the isolates were sensitive to Erythromycin, the existence of resistant isolates should not be ruled out because several isolates showed resistance to this antibiotic. The present study showed that 100% of isolates were sensitive to Gentamicin. The widespread occurrence of antibiotic resistance within *Arcobacter* spp. across various settings could potentially contribute to the dissemination of resistance. It is crucial to collect information on antimicrobial resistance because these drugs are often recommended as the first treatment for infection. Also, data about the antibiotic sensitivity obtained in this study can be used during the design of the study for the isolation of these bacteria. This study showed that the susceptibility of *A. butzleri* to different types of antibiotics is variable.

According to the results of the present study, *A. butzleri* was found in different environmental samples, poultry meat, beef, sheep meat, and the sewage of slaughterhouses. Assessment of antibiotic resistance in the isolates from this study showed that there is relatively high antibiotic resistance and multiple antibiotic resistance in the majority of the isolates. Methods such as microarray expression profiles and proteomic investigations can provide a more comprehensive insight into *Arcobacter* infection.

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Conflict of interest

The authors declare that they have no conflict of interests.

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