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The Investigation of the Presence and Antimicrobial Profiles of *Arcobacter* Species in Sheep Carcasses and Feces

Cansu ÇELİK[1], Serkan İKİZ[2]

[1]Department of Food Processing, Food Technology Programme, İstanbul University-Cerrahpaşa, Vocational School of Veterinary Medicine, İstanbul, Turkey

[2]Department of Microbiology, İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, İstanbul, Turkey

Cite this article as: Çelik, C., İkiz, S., 2019. The Investigation of the Presence and Antimicrobial Profiles of *Arcobacter* Species in Sheep Carcasses and Feces. Acta Vet Eurasia 45, 42-49.

ORCID IDs of the authors: C.Ç. 0000-0002-9508-7473; S.İ. 0000-0001-6502-0780.

Abstract

This study was designed to investigate the presence and the prevalence of *Arcobacter butzleri, Arcobacter cryaerophilus,* and *Arcobacter skirrowii* in sheep carcass swabs and feces from sheep with and without diarrhea raised in Istanbul. Also it was aimed to determine their resistance profiles against some antimicrobials. For this purpose, 50 fecal samples from sheep without diarrhea, 50 fecal samples from sheep with diarrhea, and 50 carcass swab samples from sheep were the material of this study. *Arcobacter* spp. were isolated from 49 (32.6%) of total 150 samples. 34 samples (68%) of 50 fecal samples from sheep with diarrhea, 5 samples (10%) from 50 fecal samples of sheep without diarrhea and 10 samples (20%) of 50 sheep carcass swabs were found to be positive according to isolation results. According to multiplex Polymerase Chain Reaction (mPCR) results, 31 of 49 were identified as *A. skirrowii* (63.3%), 9 of 49 were *A. butzleri* (18.3%), while 9 of 49 were *A. cryaerophilus* (18.3%). As a conclusion, *Arcobacter* species should be taken into consideration especially in sheep with diarrhea. Additionally, it should be considered that arcobacters have started to gain resistance against fluoroquinolones.

Keywords: *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii,* antibiotic sensitivity test, mPCR, sheep

Introduction

The importance of arcobacters that are described as foodborne and zoonotic entero-pathogens has increased in recent years. *Arcobacter* species (spp.) are considered emerging food-borne entero-pathogens (Abay et al., 2012; Atabay and Corry, 1998). Although it varies among species, these bacteria can be found in animals showing signs of gastroenteritis, abortion and mastitis whereas it can also be seen in healthy animals which do not have these symptoms (On et al., 2002, Vandamme et al., 1992b). Especially *Arcobacter butzleri (A. butzleri)* is the most known species that they can cause food-borne gastroenteritis and septicemia in human beings (Lau et al., 2002; Vandamme et al., 1992b).

Lately, *Arcobacter* spp. has been frequently isolated from animal source foods (Fernandez et al., 2015; Sekhar et al., 2017). Although they have shown significant phenotypic heterogeneity in terms of their biochemical and physiological characteristics, the genus *Arcobacter* is classified together with *Campylobacter* genus under the *Campylobacteriaceae* family (Vandamme and De Ley, 1991). Their ability to grow at temperatures below 30°C and aerotolerance characters are the features that seperate *Arcobacter species* from *Campylobacter* species. Therefore, they have been defined as "aerotolerant campylobacters" (Neill et al., 1978).

Arcobacters have been identified as a potential zoonotic agent of food and water origin. The discovery of new species has enlarged the genus. Recently, the whole *Arcobacter* genus has been defined to contain 25 species (Ramees et al., 2017). In the genus, *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* can more probably cause human diseases (Assanta et al., 2002). Among several *Arcobacter* species, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* are reported to have veterinary and public health sig-

Address for Correspondence: Cansu ÇELİK • **E-mail:** cansu.celik@istanbul.edu.tr

Received Date: 25 July 2018 • **Accepted Date:** 05 March 2019 • DOI: 10.26650/actavet.2019.18007

Available online at actaveteurasia.istanbulc.edu.tr

nificance. *Arcobacter* spp. have been included among microorganisms that pose a high risk for human health and *A. butzleri* can be isolated from many human cases, it has been accepted as the most significant species in this genus by International Food Microbiology Specification Committee (Collado and Figueras, 2011).

Although there are many medium and different procedures for the identification of *Arcobacter* spp., no standard reference methods have been suggested. Atabay and Corry (1998) used an arcobacter broth with the addition of cefoperazone, amphotericin, and teicoplanin (CAT) supplements in their research, and more recently, Houf et al. (2001) developed an *Arcobacter*-specific isolation method with the use of an arcobacter medium. This medium consisted of five antibiotics, including cefoperazone, trimethoprim, amphotericin, novobiocin, and 5-fluorouracil.

Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) with the addition of a CAT supplement was used by Kemp et al. (2005). Columbia agar containing 5% (vol/vol) of defibrinated horse blood was also used by Merga et al. (2011).

The biochemical properties of *Arcobacter* spp. reported by Vandamme et al. (1992a) are that *A. butzleri, A. cryaerophilus, and A. skirrowii* strains have oxidase and catalase activities and can not produce hydrogen sulfide on Triple Sugar Iron agar.

Methods used for direct detection and/or typing of the species in the genus include PCR, multiplex PCR (mPCR), real time PCR (RT-PCR), fluorescence *in situ* hybridisation (FISH), denaturing gradient gel electrophoresis PCR (DGGE-PCR), restriction fragment length polymorphism (RFLP) and matrix assisted laser desorption ionization mass spectrometry (MALDITOF MS) Enterobacterial repetitive intergenic consensus (ERIC) PCR was set up and optimized for the characterization of *A. butzleri, A. cryaerophilus, and A. skirrowii* strains (Houf et al., 2002).

Recently, mPCR has been developed as a very useful method for quick identification of *Arcobacter* species. It has been reported that there is no risk of false positive results based on the contamination with other bacteria from *Campylobacteriaceae* family. It can reduce the time required for the identification of *Arcobacter* spp. and eliminate the likelihood of false-positive results because of other *Campylobacteriaceae* family members (Neubauer and Hess, 2006; Snelling et al., 2006).

Fluroquinolones and tetracycline have been suggested for the treatment of infections caused by *A. butzleri* in humans and animals (Son et al., 2007; Vandenberg et al., 2006). It is stated that the resistance to erythromycin and the susceptibility to ciprofloxacin may have human health effects, as the two antimicrobials are generally used as first-line drugs to treat the bacterial infections due to bacteria in the *Campylobacteriaceae* family (Rahimi, 2014).

Abay et al. (2012) argued as a conclusion of their study that gentamycin, streptomycin, and tetracycline would be suitable antibiotics for the treatment or control of the disease caused by *Arcobacter* spp. in veterinary and human medicine. Aski et al. (2016) reported that all *Arcobacter* isolates were resistant to rifampicin, vancomycin, ceftriaxone, trimethoprim, and cephalothin, and furthermore, the isolates showed high susceptibility to tetracycline, oxytetracycline, erythromycin, ciprofloxacin, kanamycin, amikacin, gentamicin, and enrofloxacin.

This study aimed to investigate the presence and the prevalence of *A. butzleri, A. cryaerophilus,* and *A. skirrowii* in sheep carcass swabs and feces of sheep with and without diarrhea. It also aimed to determine their resistance profiles against some antimicrobials. In addition, the possible effects of season, gender, sample type, and age on the prevalence of arcobacters were examined.

Materials and Methods

This study was approved by Ethic Committee of the İstanbul University Faculty of Veterinary Medicine (Approval number: 2013/74).

Samples

Fifty fecal samples from healthy sheep, 50 fecal samples from sheep with diarrhea, and 50 swab samples from sheep carcasses were taken from different farms and slaughterhouses located in İstanbul, Turkey. All the samples were gathered in the same year and only one sample was received from each sheep. 150 samples were taken from 73 male and 77 female animals. The details of the samples and their collections were given in Table 1. The fecal samples were collected from rectum and stored in sterile plastic containers and the carcass swab samples were taken by using sterile cotton swabs. The swab samples were taken from the area from neck to the hips (50 cm^2) according to the EU Regulation 2073/2005. All the samples were analyzed in the microbiology laboratory in 3 hours after they were collected from the sheep.

Reference strains

The positive control strains belong to *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* were taken from Victor Segalen Bordeaux II University Hospital's Bacteriological Laboratory.

Culture

Two grams of fecal materials were transferred into the test tubes including 9 ml of arcobacter broth (Oxoid, Basingstoke, UK), and each swab sample was transferred into the test tubes including 2 ml of arcobacter broth with the addition of 5-fluorouracil (100 mg/1000 mL) (Merck, Darmstadt, Germany), amphotericin B (10 mg/1000 mL) (Bioshop, Ontario, Canada), cefoperazone (16 mg/1000 mL) (Sigma-Aldrich, Saint Louis, USA), novobiocin (32 mg/1000 mL) (Sigma-Aldrich, Saint Louis, USA), and trimethoprim (64 mg/1000 mL) (Sigma-Aldrich, Saint Louis, USA) (Houf et al., 2001) as an enrichment broth, and were incubated aerobically

Table 1. The information regarding the samples collected in one year

Sample type (n:number)	Farm code	Sample number	The month of sample collection
Fecal samples from sheep without diarrhea (n=50)	FARM A	20	MAY
	FARM B	20	JUNE, JULY
	FARM C	10	AUGUST
Fecal samples from sheep with diarrhea (n=50)	FARM A	3	MAY
	FARM D	6	SEPTEMBER
	FARM E	10	OCTOBER
	FARM F	4	NOVEMBER
	FARM G	14	NOVEMBER
	FARM H	13	DECEMBER
Carcass swab samples (n=50)	SLAUGHTERHAUSE A	10	JULY
	SLAUGHTERHAUSE B	15	JULY
	SLAUGHTERHAUSE C	15	AUGUST
	SLAUGHTERHAUSE D	10	OCTOBER

Table 2. Primer sequences used in this study (Houf et al., 2000)

	Primers	Sequence (5' to 3')
A.butzleri	BUTZ	CCT GGA CTT GAC ATA GTA AGA ATGA
16Ss r DNA	ARCO	CGT ATT CAC CGT AGC ATA GC
A.skirrowii	SKIR	GGC GAT TTA CTG GAA CAC A
A.cryaerophilus	CRY1	TGC TGG AGC GGA TAG AAG TA
23S rDNA	CRY2	AAC AAC CTA CGT CCT TCG AC

at 30°C for 24 h. At the same time, motility tests were performed with each sample taken from arcobacter broth after 24 h as soon as the pre-enrichment stage was completed. Spiral or corkscrew motility types were observed. After the pre-enrichment phase, 15 µl was collected from broth culture and was inoculated onto modified charcoal-cefoperazone-deoxycholate agar (Scharlab, Barcelona, Spain) as a selective medium which consisted of CAT supplement (Oxoid, Basingstoke, UK).

It was incubated for 48 h at 30°C under aerobic conditions. At the end of incubation, Gram staining was performed on gray-white, small, round, and S-type colonies. Gram negative colonies were transferred to Columbia agar (Biolife, Milan, Italy). The Gram staining properties of gray-white, small, round, and S-type colonies were reexamined after 24-48 h (Merga et al., 2011). The biochemical features of gram negative bacteria showed catalase and oxidase activities, but no production of hydrogen sulfide was detected from any of the strains. Additionally all reference strains were isolated with the same method.

Multiplex polymerase chain reaction (mPCR)

The isolates were then identified to species level by multiplex PCR (Houf et al., 2000). A boiling method was used to extract DNA from the *Arcobacter* isolates.

Selected primer sequences shown in Table 2 were amplified according to the method by Houf et al. (2000) through PCR, using a reaction mixture containing 4 µl 10X PCR buffer+$MgCl_2$, 0.8 µl 10mM dNTPs, 2 µl (20 pmol/mL) of each of the four primers (BUTZ, ARCO, CRY1, CRY2) and 1 µl (20 pmol/mL) of SKIR primer, 0.3 µl of Taq DNA polymerase, 15.9 µl DNase-free water, and 10 µl bacterial DNA for each sample. PCR amplification kit (Qiagen, Germantown, USA) was used in the study. The reactions were performed in a thermal cycler (Biometra UNO-Thermoblock, Dublin, Ireland) with the following amplification conditions: a denaturation step for 3 min at 94°C; 37 amplification cycles: denaturation for 2 min at 94°C, annealing for 45 sec at 61°C, and for 30 sec. extension at 72°C; the final extension step was 7 min at 72°C.

The electrophoresis (Biometra, Dublin, Ireland) was performed approximately at 125V for 45 min. The bands were visualized on an UV illuminator. The resulting products sizes were 257 bp for *A. cryaerophilus*, 401 bp for *A. butzleri,* and 641 bp for *A. skirrowii*. Furthermore, all reference strains were identified with the same technique.

Antibiotic sensitivity test

The antibiotic resistance profiles of *Arcobacter* strains were determined by using disc diffusion technique. Blood agar that comprised 5% (v/v) of defibrinated horse blood in blood agar base no. 2 was used for this purpose. Sterile cotton-tipped swab was used for spreading on the plate. Later on, each antibiotic disc was placed onto the agar and the plates were kept at 4°C for about 15 min. The plates incubated in a micro-aerobic atmosphere at 30°C for 48 h and the diameters of the inhibition zones were measured with calipers. The diameters of the zones occurred around the discs were evaluated according to the criteria defined for materials for *Campylobacter* spp. by the National Committee for Clinical Laboratory Standards and also

according to the criteria specified for *Enterobacteriaceae* by the European Committee on Antimicrobial Susceptibility Testing (2015), since no standardized source was described yet to make comparison.

Statistical analysis

In order to determine the statistical significance of the findings, the results were evaluated with "Chi-square (x^2) test" by SPSS 13.0 programme (SPSS Inc.; IL, Chicago, USA) by comparing the positive rates of *A. butzleri, A. cryaerophilus,* and *A. skirrowii* under sex, age, season, and sample subgroups.

Results

Culture and isolation studies

A total of 150 samples were collected from 50 sheep with diarrhea, 50 healthy sheep, and 50 sheep carcasses from the slaughterhouses and farms in and around Istanbul province. Fourty-nine (32.6%) of 150 samples were detected as *Arcobacter* spp.

Thirty-four (68%) of 50 fecal samples from sheep with diarrhea, 5 (10%) of 50 fecal samples from sheep without diarrhea and 10 (20%) of 50 sheep carcass swab samples were found to be positive according to isolation results.

Multiplex Polymerase Chain Reaction (mPCR)

Presumptive colonies were chosen according to their macroscobic and microscobic features. Only one suspicious colony from each culture were applied for PCR identification. A total of 49 culture positive samples were tested by mPCR and all 49 (100%) samples were positive in terms of *Arcobacter* species. As a result of the agarose gel electrophoresis of the mPCR products, 257 bp for *A. cryaerophilus*, 401 bp for *A. butzleri,* and 641 bp bands for *A. skirrowii* were detected. According to mPCR, 31 of 49 samples were identified as *A. skirrowii* (63.2%), 9 of 49 as *A. butzleri* (18.3%), while 9 of 49 as *A. cryaerophilus* (18.3%) (Figures 1, 2).

Antibiotic sensitivity testing

All strains identified as *A. butzleri* were susceptible to methicillin (30 µg) (Bioanalyse, Ankara, Turkey) and gentamycin (10 µg)

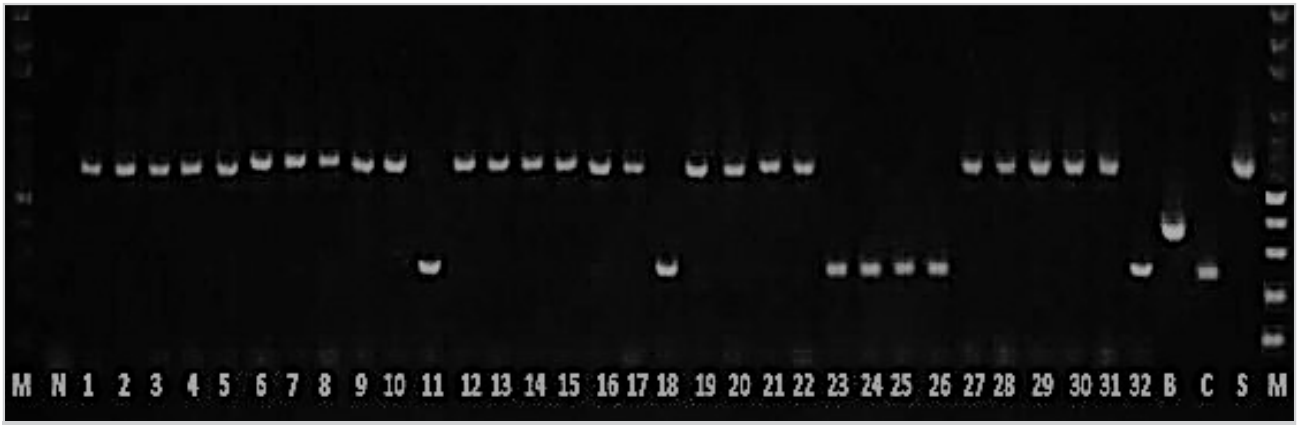

Figure 1. M: Marker, N: Negative Control, B: *A.butzleri* Positive Control, C: *A.cryaerophilus* Positive Control, S: *A.skirrowii* Positive Control, 1-32: Samples

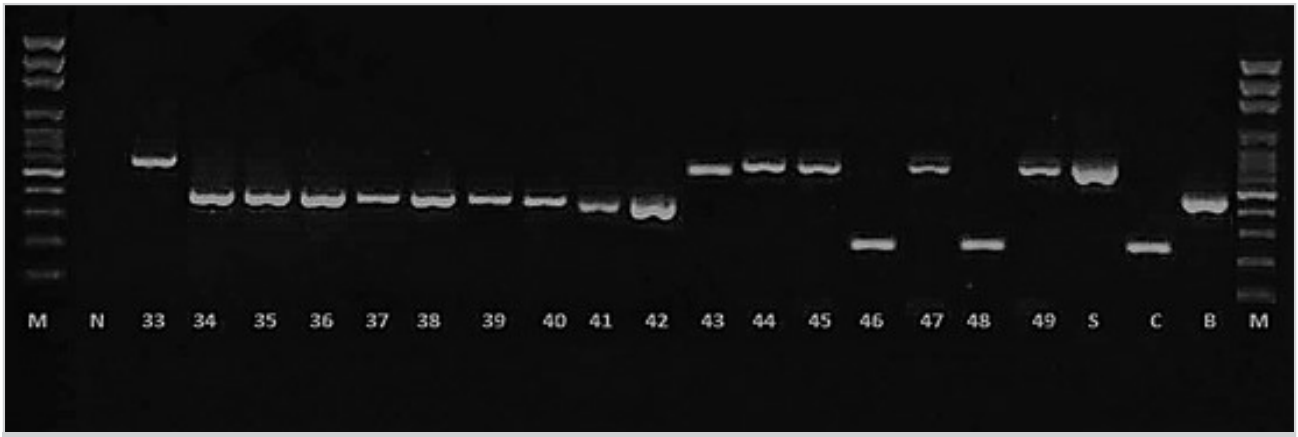

Figure 2. M: Marker, N: Negative Control, B: *A.butzleri* Positive Control, C: *A.cryaerophilus* Positive Control, S: *A.skirrowii* Positive Control, 33-49: Samples

Table 3. Antibiotic sensitivity test results of *Arcobacter* strains

		***A.butzleri* (n)**			***A.cryaerophilus* (n)**			***A.skirrowii* (n)**		
	Antimicrobial Agent	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**
Penicillin	Penicillin G 10 unit/disk	9	0	0	9	0	0	29	0	2
	Ampiciline + Sulbactam 1:120 mg	3	3	3	5	3	1	5	14	12
	Amoxicillin 25 µg	2	5	2	5	3	1	11	12	8
	Methicillin 30 µg	0	9	0	8	0	1	17	4	10
	Amoxicillin + Clavulanic acid 2:1 30 µg	3	5	1	4	4	1	10	21	0
Tetracyclines	Tetracycline 30 µg	2	6	1	0	9	0	0	31	0
	Oxytetracycline 30 µg	4	5	0	0	7	2	0	25	6
	Doxycycline 30 µg	1	6	2	0	9	0	1	30	0
Quinolones	Ofloxacin 5 µg	8	1	0	9	0	0	9	5	17
	Ciprofloxacin 5 µg	4	0	5	4	1	4	7	6	18
	Enrofloxacin 5 µg	2	2	5	3	3	3	2	22	7
	Nalidixic Acid 30 µg	9	0	0	6	1	2	8	8	15
Aminoglycosid	Amikacin 30 µg	0	7	2	0	9	0	1	29	1
	Gentamisin 10 µg	0	9	0	0	8	1	2	28	1
Macrolides	Erythromycin 15 µg	0	0	9	0	1	8	1	0	30
Rifamycin	Rifampicin 30 µg	9	0	0	9	0	0	30	1	0
Cephalosporins	Cephalothin 30 µg	8	1	0	8	0	1	28	1	2
Nitrofurantoin	Nitrofurantoin 300 µg	8	1	0	4	4	1	1	30	0
Vancomycin	Vancomycin 30 µg	9	0	0	9	0	0	29	1	1

R: resistant; S: sensitive; I: intermediate

(Oxoid, Basingstoke, UK), and all *A. cryaerophilus* strains were susceptible to tetracycline (30 µg) (Oxoid, Basingstoke, UK), doxycycline (30 µg) (Oxoid, Basingstoke, UK), and Amikacin (30 µg) (Oxoid, Basingstoke, UK), while all *A. skirrowii* strains were found to be susceptible to tetracycline.

All of *A. butzleri* strains had intermediate sensitivity to erythromycin (15 µg) (Oxoid, Basingstoke, UK), whereas all *A. butzleri* strains had resistance to penicillin G (10 Unit/disk) (Oxoid, Basingstoke, UK), rifampicin (30 µg) (Oxoid, Basingstoke, UK), vancomycin (30 µg) (Oxoid, Basingstoke, UK), and nalidixic acid (30 µg) (Oxoid, Basingstoke, UK). And all *A. cryaerophilus* strains showed resistance to ofloxacin (5 µg) (Oxoid, Basingstoke, UK), vancomycin, and rifampicin. Other strains showed variable results as shown in Table 3.

Statistical findings

The effects of season, age, gender, and sample type on isolation rate were examined. The effect of season was found statistically significant ($p<0.05$) for *A. cryaerophilus* and *A. skirrowii`s* isolation rates. The effect of age was statistically significant ($p<0.05$) on *A. butzleri* and *A. skirrowii*'s isolation rates. The effect of gender was not statistically significant for none of these three *Arcobacter* species` isolation ratios. The sample type was found 99% statistically significant ($p<0.05$) related to *A. butzleri, A. cryaerophilus,* and *A. skirrowii`s* isolation rates.

Discussion

Arcobacters have been accepted as "important zoonotic pathogenic" bacterial strains (Cardoen et al., 2009). The non-human sources of arcobacters have also been reported to be healthy or have affected animals of various species, various foods, and water (Kabeya et al., 2003).

In a study carried out in Turkey (Sürmeli, 2006), 104 sheep stool samples were taken under microaerobic conditions using membrane filtration technique and only one *A. cryeraophilus* was identified by mPCR method as reported. In Belgium, Van Driessche et al. (2003) collected 62 healthy sheep feces from slaughterhouses and performed a direct isolation from the feces samples. According to their report, they identified *A. butzleri* only in 3 (4.8%) samples, whereas 10 *Arcobacter* species (16%) were isolated after pre-enrichment.

In our study, 31 of 49 were identified as *A. skirrowii* (63.3%), 9 of 49 as *A. butzleri* (18.3%), while 9 of 49 as *A. cryaerophilus* (18.3%). Using the arcobacter broth medium-mCCDA-Columbia agar (defibrinated horse blood supplement) as an isolation technique may account for the increase in the isolation rate. Another explanation for the differences found in the rates may be the use of stool sample instead of swab. In this study, 8 *A. butzleri* (16%), 1 *A. cryaerophilus* (2%), and 1 *A. skirrowii* (2%)

were identified from 50 sheep carcass swab samples. Any information with regard to whether the animals having enteritis could not be obtained.

In a study carried out in Turkey (Ertaş and Doğruer, 2009), *Arcobacter* spp. were isolated from 85 (42.5%) of 200 minced meat samples. *A. butzleri* was detected from 39% and *A. skirrowii* was detected from 4% of minced sheep meat samples, while *A. butzleri* was detected from 40% and *A. skirrowii* was detected from 2% of minced cattle meat. The prevalence rate of arcobacters in minced meat obtained from sheep in their study was higher than the prevalence rate we had from swab sample in our thesis study. The difference in prevalence suggested a possible increase in *Arcobacter* contamination as the meat samples were processed by equipment like mincer. Further, the microorganisms on the surface of the meat might have spread all over it during the course of grinding and mixing the mincemeat leading to food spoilage.

In Japan, Kabeya et al. (2003) found 12 (3.6%) *Arcobacter* species from 332 healthy cattle feces samples, whereas Van Driessche et al. (2003) identified 39.2% *Arcobacter* species from healthy bovine feces samples and 16.1% *Arcobacter* species from healthy ovine feces samples. Nachamkin et al. (2008) reported that they identified *A. butzleri* from feces samples of pig, cattle, horse, ostrich, turtle, and *A. skirrowii* from sheep and cattle with diarrhea and hemorrhagic colitis. *A. skirrowii* was detected from sheep having enteritis and most *A. butzleri* strains were isolated from diarrheic feces of humans and animals (Vandamme et al., 1992a). In our study, 34 (68%) *Arcobacter* species were detected from 50 feces samples belonged to sheep with enteritis and we found 25 (50%) *A. skirrowii*, 8 (16%) *A. cryaerophilus,* and 1 (2%) *A. butzleri*.

It was reported that season, climate, geographical location, sampling type, and isolation method had an effect on the prevalence values to be obtained (Merga et al., 2011). Golla et al. (2002) stated that there was a direct correlation between increased age and increased prevalence of arcobacters in their studies. In our study, the incidence of *A. cryaerophilus* increased in proportion to the increase in age from 1 month to 3 years, but the graph showed a reverse slope for *A. butzleri*. The incidence of *A. skirrowii* reached the highest rate between 1 and 3 years.

Researchers reported that there was no significant difference in the rates of arcobacters between goats and sheep ($p>0.05$) (Van Driessche et al., 2003; Van Driessche et al., 2005). In our study, the effect of gender difference on the incidence of *Arcobacter* species was statistically insignificant ($p>0.05$). The effect of seasons on the incidence of *A. cryaerophilus* ($p<0.05$) and *A. skirrowii* ($p<0.001$) in this study was also statistically significant.

It was also reported in other research (Van Driessche et al., 2003; 2005), as reported in this study, that the difference in season and farm management could have an effect on the rates found. The heavy conditions of the winter season in Turkey and keeping the animals in narrow and unhygienic shelters may be another cause of the seasonal effect. The effect of the presence of diarrhea was found to be statistically significant on the isolation rates ($p<0.001$) of *A. cryaerophilus* ($p<0.01$) and *A. skirrowii*.

The most commonly prescribed drugs as antibiotics are erythromycin or a fluoroquinolone such as ciprofloxacin (Luber et al., 2003). Tetracycline, doxycycline, and gentamicin are sometimes listed as alternative drugs for treatment (Houf et al., 2004). Pérez-Cataluña et al. (2017) stated that the resistance to ciprofloxacin, one of the antibiotics recommended for the treatment of intestinal infections of *Arcobacter*, detected 10.7% of the strains, and indicated the importance of selecting the most effective treatment. In our study as well, most of the *Arcobacter* strains showed a resistance or an intermediate profile against some of the fluoroquinolones antibiotics, such as enrofloxacin, ofloxacin, and ciprofloxacin. The resistance to erythromycin and quinolone antibiotics found in this study can be considered as a worrisome condition, because these antimicrobial agents have been reported to be the first-line antibiotics used in the treatment of infections caused by members of the Campylobacteracea family (Houf et al., 2004).

In Milesi's research (2011), it was reported that all *Arcobacter* spp. isolates from animal originated food were detected as resistant to cephalothin, sulfamethoxazole / trimethoprim, and nalidixic acid. The majority of *A. cryaerophilus* and *A. skirrowii* strains were found as sensitive to tetracyline and amikacin, which is compatible with some other research results (Abay et al., 2012; Collado and Figueras, 2011; Son et al., 2007; Ünver et al., 2013). However, *A. butzleri*'s strains were detected to have started to gain some resistance against to these 2 antimicrobial agents.

The results have exhibited that *A. skirrowii* may also show a significant diarrhea effect in sheep, such as in human and other animals. Considering the increase in the prevalence of *Arcobacter spp.* isolates from sheep in autumn (Grove-White et al., 2014), another reason for the high incidence of *A. skirrowii* isolates in our research may be the gathering of diarrhea cases in November and December.

Although the results of the studies on fluoroquinolones indicate that it is the most effective antibiotic group in the treatment of *Arcobacter* infections, most of the *Arcobacter* strains detected in this research show resistance or half sensitivity to enrofloxacin, ofloxacin, and ciprofloxacin, which are the antibiotics of this group. In this study arcobacters cause diarrhea in sheep and these animals may play a very important reservoir role for humans. Hence, as with human beings, of the analyses of diarrhea cases should take into consideration the existence of *Arcobacter* species in sheep. As a result, the study suggests, in line with many other researches, that these varying sensitivity and resistance profiles to the antibiotics may be due to the

lack of a standard antimicrobial susceptibility test developed for *Arcobacter* species (Vandenberg et al., 2006).

In conclusion, *A. skirrowii A. butzleri* and *A. cryaerophilus* were identified with the rate of (63.3%), (18.3%) and (18.3%) respectively. *A. skirrowii* was found more than other *Arcobacter* species in fecal samples with diarrhea. Regarding the effect of season, age, sample type and gender, only the effect of gender was not found statistically significant for none of these three *Arcobacter* species` isolation ratios whereas the effect of season was found statistically significant for *A. cryaerophilus* and *A. skirrowii`s* isolation rates and the effect of age was statistically significant on *A. butzleri* and *A. skirrowii*'s isolation rates. The sample type was found also statistically important regarding *A. butzleri, A. cryaerophilus,* and *A. skirrowii`s* isolation rates. In terms of the medication of *Arcobacter* infections, it was observed that they started to gain resistance against to fluoroquinolones which was known as the best antibiotic groups for arcobacters.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University Faculty of Veterinary Medicine (2013/74).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.İ., C.Ç.; Design – S.İ., C.Ç.; Supervision – S.İ.; Resources – C.Ç.; Materials – C.Ç.; Data Collection and/or Processing – C.Ç.; Analysis and/or Interpretation – C.Ç., S.İ.; Literature Search – C.Ç.; Writing Manuscript – C.Ç.; Critical Review – C.Ç., S.İ.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This study was supported by Scientific Research Projects Coordination Unit of İstanbul University (Project number: 35968).

References

Abay S., Kayman T., Hizlisoy H., Aydin F., 2012. In vitro antibacterial susceptibility of *Arcobacter butzleri* isolated from different sources. Journal of Veterinary Medical Science 74, 613-616. [CrossRef]

Aski H.S., Tabatabaei M., Khoshbakht R., Raeisi M., 2016. Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. Comparative Immunology, Microbiology and Infectious Diseases 44, 37-40. [CrossRef]

Assanta M.A., Roy D., Lemay M.J., Montpetit D., 2002. Attachment of *Arcobacter butzleri*, a new waterborne pathogen, to water distribution pipe surfaces. Journal of Food Protection 65, 1240-1247. [CrossRef]

Atabay H.I., Corry, J.E., 1998. Evaluation of a new arcobacter enrichment medium and comparison with two media developed for enrichment of *Campylobacter* spp. International Journal of Food Microbiology 41, 53-58. [CrossRef]

Cardoen S., Van Huffel X., Berkvens D., Quoilin S., Ducoffre G., Saegerman C., Speybroeck N., Imberechts H., Herman L., Ducatelle R., Dierick K., 2009. Evidence-based semiquantitative methodology for prioritization of foodborne zoonoses. Foodborne Pathogens and Disease 6, 1083-1095. [CrossRef]

Collado L., Figueras M.J., 2011. Taxonomy, epidemiology and clinical relevance of the genus *Arcobacter*. Clinical Microbiology Reviews 24, 174-192. [CrossRef]

Ertaş N., Doğruer Y., 2009. Prevalence of *Arcobacter* species in ground meat from cattle and sheep using multiplex PCR techniques. Fırat University Medical Journal of Health Sciences 23, 95-100.

European Committee on Antimicrobial Susceptibility Testing, 2015. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for Interpretation of MICs and zone diameters ,Version 5.0

Fernandez H., Villanueva M.P., Mansilla I., Gonzalez M., Latif F., 2015. *Arcobacter butzleri* and *A. cryaerophilus* in human, animals and food sources, in southern Chile. Brazilian Journal of Microbiology 46, 145-147. [CrossRef]

Golla S.C., Murano E.A., Johnson L.G., Tipton N.C., Cureington E.A., Savell J.W., 2002. Determination of the occurrence of *Arcobacter butzleri* in beef and dairy cattle from Texas by various isolation methods. *Journal of Food Protection* 65, 1849-1853. [CrossRef]

Grove-White D.H., Leatherbarrow A.J.H., Cripps P.J., Diggle P.J., French N.P., 2014. Temporal and farm-management-associated variation in faecal pat prevalence of *Arcobacter* spp. in ruminants. Epidemiology & Infection 142, 861-870. [CrossRef]

Houf K., Devriese L.A., De Zutter L., Van Hoof J., Vandamme P., 2001. Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry products. International Journal of Food Microbiology 71, 189-196. [CrossRef]

Houf K., Devriese L.A., Haesebrouck F., Vandenberg O., Butzler J.P., Hoof J.V., Vandamme P., 2004. Antimicrobial susceptibility patterns of *Arcobacter butzleri* and *Arcobacter cryaerophilus* strains isolated from humans and broilers. Microbial Drug Resistance 10, 243-247. [CrossRef]

Houf K., De Zutter L., Van Hoof J., Vandamme P., 2002. Assessment of the genetic diversity among Arcobacters isolated from poultry products by using two PCR- based typing methods. Applied Enviromental Microbiology 68, 2172-2178. [CrossRef]

Houf K., Tutenel A., de Zutter L., Hoof J.V., Vandamme P., 2000. Development of a multiplex PCR assay for simultaneous detection and identification *of Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiology Letters 193, 89-94. [CrossRef]

Kabeya H., Maruyama S., Morita Y., Kubo M., Yamamoto K., Arai S., Izumi T., Kobayashi Y., Katsube Y., Mikami T., 2003. Distribution of *Arcobacter* species among livestock in Japan. Veterinary Microbiology 93, 153-158. [CrossRef]

Kemp R., Leatherbarrow A.J., Williams N.J., Hart C.A., Clough H.E., Turner J., Wright E.J., French N.P., 2005. Prevalence and genetic diversity of *Campylobacter* spp. in environmental water samples from a 100-square-kilometer predominantly dairy farming area. Applied and Environmental Microbiology 71, 1876-1882. [CrossRef]

Lau S.K., Woo PC, Teng J.L., Leung K.W., Yuen K.Y., 2002. Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient wit[CrossRef]

Luber P., Bartelt E., Genschow E., Wagner J., Hahn H., 2003. Comparison of broth microdilution, E Test, and agar dilution methods for an-

tibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. Journal of Clinical Microbiology 41, 1062-1068. [CrossRef]

Merga J.Y., Leatherbarrow A.J., Winstanley C., Bennett M., Hart C.A., Miller W.G., Williams N.J., 2011. Comparison of *Arcobacter* isolation methods, and diversity of *Arcobacter* spp. in Cheshire, United Kingdom. Applied and Environmental Microbiology 77, 1646-1650. [CrossRef]

Milesi, S., 2011. Emerging pathogen *Arcobacter* spp. in food of animal origin. Doctoral thesis, University of Milan, Milan.

Nachamkin, I., Szymanski, C. M., Blaser, M. J., 2008. Campylobacter (No. Ed. 3). ASM Press.

Neill S.D., Ellis W.A., O'Brien J.J., 1978. The biochemical characteristics of *Campylobacter*-like organisms from cattle and pigs. Research in Veterinary Science 25, 368-372. [CrossRef]

Neubauer C., Hess M., 2006. Detection and identification of foodborne pathogens of the genera *Campylobacter, Arcobacter* and *Helicobacter* by multiplex PCR in poultry and poultry products. Journal of Veterinary Medicine Series B 53, 376-381. [CrossRef]

On S.L., Jensen T.K., Bille-Hansen V., Jorsal S.E., Vandamme P., 2002. Prevalence and diversity of *Arcobacter* spp. isolated from the internal organs of spontaneous porcine abortions in Denmark. Veterinary Microbiology 85, 159-167. [CrossRef]

Pérez-Cataluña A., Tapiol J., Benavent C., Sarvisé C., Gómez F., Martínez B., Terron-Puig M., Recio G., Vilanova A., Pujol I., Ballester F., 2017. Antimicrobial susceptibility, virulence potential and sequence types associated with *Arcobacter* strains recovered from human faeces. Journal of Medical Microbiology 66, 1736-1743. [CrossRef]

Rahimi E., 2014. Prevalence and antimicrobial resistance of *Arcobacter* species isolated from poultry meat in Iran. British Poultry Science 55, 174-180. [CrossRef]

Ramees T.P., Dhama K., Karthik K., Rathore R.S., Kumar A., Saminathan M., Tiwari R., Malik Y.S., Singh R.K., 2017. *Arcobacter*: an emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control–a comprehensive review. Veterinary Quarterly 37, 136-161. [CrossRef]

Sekhar, M.S., Tumati, S.R., Chinnam, B.K., Kothapalli, V.S., Sharif, N.M., 2017. Virulence gene profiles of *Arcobacter* species isolated from animals, foods of animal origin, and humans in Andhra Pradesh, India. Veterinary World 10, 716. [CrossRef]

Snelling W.J., Matsuda M., Moore J.E., Dooley J.S., 2006. Under the microscope: *Arcobacter*. Letters in Applied Microbiology 42, 7-14. [CrossRef]

Son I., Englen M.D., Berrang M.E., Fedorka-Cray P.J., Harrison M.A., 2007. Antimicrobial resistance of *Arcobacter* and *Campylobacter* from broiler carcasses. International Journal of Antimicrobial Agents 29, 451-455. [CrossRef]

Sürmeli P., 2006. Kars ili ve çevresinde yetiştirilen koyunlardan alınan dışkı örneklerinde *Arcobacter* türlerinin prevalansının araştırılması. Master thesis, Kafkas University, Faculty of Veterinary Medicine, Department of Microbiology, Institute of Health Sciences, Kars.

Ünver A., Atabay H.I., Şahin M., Çelebi Ö., 2013. Antimicrobial susceptibilities of various *Arcobacter* species. Turkish Journal of Medical Sciences 43, 548-552. [CrossRef]

Van Driessche E., Houf K., Hoof J.V., De Zutter L., Vandamme P., 2003. Isolation of *Arcobacter* species from animal feces. FEMS Microbiology Letters 229, 243-248. [CrossRef]

Van Driessche E., Houf K., Vangroenweghe F., De Zutter L., Van Hoof J., 2005. Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium. Veterinary Microbiology 105, 149-154. [CrossRef]

Vandamme P., De Ley J., 1991. Proposal for a new family, Campylobacteraceae. International Journal of Systematic and Evolutionary Microbiology 41, 451-455. [CrossRef]

Vandamme P., Pugina P., Benzi G., Van Etterijck R., Vlaes L., Kersters K., Lauwers S., 1992b. Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. Journal of Clinical Microbiology 30, 2335-2337.

Vandamme P., Vancanneyt M., Pot B., Mels L., Hoste B., Dewettinck D., Vlaes L., Van Den Borre C., Higgins R., Hommez J., Kersters K., 1992a. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. International Journal of Systematic and Evolutionary Microbiology 42, 344-356. [CrossRef]

Vandenberg O., Houf K., Douat N., Vlaes, L., Retore P., Butzler J.P., Dediste A., 2006. Antimicrobial susceptibility of clinical isolates of non-jejuni/coli campylobacters and arcobacters from Belgium. Journal of Antimicrobial Chemotherapy 57, 908-913. [CrossRef]